A Scientific Review of the
Potential Environmental Effects of
Aquaculture in Aquatic Ecosystems

Volume III

Near-field Organic Enrichment from Marine Finfish Aquaculture
D.J. Wildish, M. Dowd, T.F. Sutherland and C.D. Levings
Environmental Fate and Effect of Chemicals Associated with
Canadian Freshwater Aquaculture
R.J. Scott

Fisheries and Oceans Canada
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FOREWORD

Context

The Government of Canada is committed to ensuring the responsible and sustainable development of the aquaculture industry in Canada. The Minister of Fisheries and Oceans' announcement of the $75 M Program for Sustainable Aquaculture (PSA), in August 2000, is a clear expression of this commitment. The objective of the PSA is to support the sustainable development of the aquaculture sector, with a focus on enhancing public confidence in the sector and on improving the industry's global competitiveness. Ensuring the sector operates under environmentally sustainable conditions is a key federal role.

As the lead federal agency for aquaculture, Fisheries and Oceans Canada (DFO) is committed to well-informed and scientifically-based decisions pertaining to the aquaculture industry. DFO has an ongoing program of scientific research to improve its knowledge of the environmental effects of aquaculture. The department is also engaged with stakeholders, provinces and the industry in coordinating research and fostering partnerships. As a contribution to the Federal government's Program for Sustainable Aquaculture, DFO is conducting a scientific review of the potential environmental effects of aquaculture in marine and freshwater ecosystems.

Goal and Scope

Known as the State-of-Knowledge (SOK) Initiative, this scientific review provides the current status of scientific knowledge and recommends future research studies. The review covers marine finfish and shellfish, and freshwater finfish aquaculture. The review focuses primarily on scientific knowledge relevant to Canada. Scientific knowledge on potential environmental effects is addressed under three main themes: impacts of wastes (including nutrient and organic matter); chemicals used by the industry (including pesticides, drugs and antifoulants); and interactions between farmed fish and wild species (including disease transfer, and genetic and ecological interactions).

This review presents potential environmental effects of aquaculture as reported in the scientific literature. The environmental effects of aquaculture activities are site-specific and are influenced by environmental conditions and production characteristics at each farm site. While the review summarizes available scientific knowledge, it does not constitute a site-specific assessment of aquaculture operations. In addition, the review does not cover the effects of the environment on aquaculture production.

The papers target a scientific and well-informed audience, particularly individuals and organizations involved in the management of research on the environmental interactions of aquaculture. The papers are aimed at supporting decision-making on research priorities, information sharing, and interacting with various organizations on research priorities and possible research partnerships.

Each paper was written by or under the direction of DFO scientists and was peer-reviewed by three experts. The peer reviewers and DFO scientists help ensure that the papers are up-to-date at the time of publication. Recommendations on cost-effective, targeted research areas will be developed after publication of the full series of SOK review papers.

State-of-Knowledge Series

DFO plans to publish 12 review papers as part of the SOK Initiative, with each paper reviewing one aspect of the environmental effects of aquaculture. This Volume contains two papers: Near-field organic enrichment from marine finfish aquaculture; and Environmental fate and effect of chemicals associated with Canadian freshwater aquaculture.

Further Information

For further information on a paper, please contact the senior author. For further information on the SOK Initiative, please contact the following:

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AVANT-PROPOS

Contexte

Le gouvernement du Canada est déterminé à assurer le développement responsable et durable de l'industrie aquacole au Canada. Le Programme d'aquaculture durable (PAD) de 75 millions de dollars annoncé par le ministre des Pêches et des Océans en août 2000 traduit clairement cet engagement. Ce programme vise à soutenir le développement durable du secteur aquacole, surtout en améliorant la confiance du public envers l'industrie et la compétitivité globale de celle-ci. Veiller à ce que l'industrie fonctionne dans des conditions durables sur le plan environnemental constitue une responsabilité essentielle du gouvernement fédéral.

À titre d'organisme fédéral responsable de l'aquaculture, Pêches et Océans Canada (MPO) est déterminé à prendre des décisions éclairées qui reposent sur des données scientifiques éprouvées en ce qui concerne l'industrie aquacole. Le MPO mène un programme de recherches scientifiques pour améliorer ses connaissances sur les effets de l'aquaculture sur l'environnement. Le Ministère collabore également avec des intervenants, les provinces et l'industrie à la coordination des recherches et à l'établissement de partenariats. Le MPO contribue au Programme de l'aquaculture durable du gouvernement fédéral en passant en revue la littérature scientifique qui aborde les effets possibles de l'aquaculture sur les écosystèmes marins et d'eau douce.

Objectif et portée

Désignée projet sur l'état des connaissances, cette revue de la littérature définit l'état actuel des connaissances scientifiques sur les effets de l'élevage de poissons et de mollusques en mer et de la pisciculture en eau douce et fait des recommandations de recherches futures. La revue, qui se concentre surtout sur les connaissances scientifiques applicables au Canada, les aborde sous trois thèmes principaux : les impacts des déchets (éléments nutritifs et matière organique), les produits chimiques utilisés par l'industrie (pesticides, médicaments et agents antisalissures) et les interactions entre les poissons d'élevage et les espèces sauvages (transfert de maladies et interactions génétiques et écologiques).

Cette revue présente les effets environnementaux possibles de l'aquaculture documentés dans la littérature scientifique. Les effets environnementaux des activités aquacoles dépendent du site, des conditions environnementales et des caractéristiques de production de chaque établissement aquacole. L'examen résume les connaissances scientifiques disponibles mais ne constitue pas une évaluation des activités aquacoles spécifique au site. L'examen ne porte pas non plus sur les effets de l'environnement sur la production aquacole.

Les articles sont destinés à un auditoire de scientifiques et de personnes bien informées, notamment des personnes et des organisations participant à la gestion de la recherche sur les interactions environnementales de l'aquaculture. Les articles visent à soutenir la prise de décision sur les priorités de recherche, la mise en commun de l'information et les interactions entre diverses organisations concernant les priorités de recherche et les partenariats de recherche possibles.

Rédigées par des scientifiques du MPO ou sous leur supervision, les articles ont été contrôlés par des pairs, ce qui assure qu'ils sont à jour au moment de leur publication. Après la publication de toute la série d'articles sur l'état des connaissances, des recommandations de recherches ciblées et rentables seront faites.

Série sur l'état des connaissances

Dans le cadre du projet de l'état des connaissances, le MPO prévoit publier douze articles de synthèse portant chacun sur un aspect des effets environnementaux de l'aquaculture. Le présent volume contient les deux articles suivants : Enrichissement organique à proximité des installations piscicoles en mer, et Devenir et effects environnementaux des produits chimiques utilisés en aquaculture en eau douce au Canada.

Renseignements supplémentaires

Pour de plus amples renseignements sur un article, veuillez communiquer avec son auteur principal. Pour de plus amples renseignements sur le projet de l'état des connaissances, veuillez communiquer avec :

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NEAR-FIELD ORGANIC ENRICHMENT FROM MARINE FINFISH AQUACULTURE

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EXECUTIVE SUMMARY

This paper reviews the literature on near-field organic enrichment associated with intensive marine finfish aquaculture. Fish farms are a source of suspended and dissolved organic matter, originating as fish feces, excess fish feed and net-cleaning wastes. The term "near-field" is used to differentiate between local (footprint limited) and more distant (far-field) effects, and refers to effects within the sedimentary footprint and between the population and community level. Near-field effects of mariculture are bounded by the physical limits of particulate waste dispersal and sedimentation from individual cages or farms.

PARTICLE TRANSPORT, DISPERSION AND BEHAVIOUR

To investigate the biogeochemical fate of organic matter in the benthic or pelagic ecosystem, it is necessary to understand how this material is transported and dispersed from the fish farm. Organic matter is transported from fish cages to the surrounding marine environment by the action of all types of water movements in the immediate vicinity of the cages. As this material moves, it spreads out and its concentration decreases, both by dilution and sedimentation.

Canadian coastal environments where aquaculture occurs are characterized by irregular coastlines and complex topography. Consequently, they often exhibit highly structured and quite complex flow fields. In strongly stratified marine systems, dissolved material can be effectively trapped in the upper or lower parts of the water column. In an aquaculture context, stratification can be an important factor in the dispersal of organic matter from certain farms in the inner portions of fjords in British Columbia.

Observational studies and numerical modelling are used to study transport and dispersion in the coastal zone. Observational studies of mixing often rely on the deployment of drifting buoys that mimic the movement of water parcels. Field

experiments releasing coloured dye into the ocean are better able to characterize dispersion from a point source, but these are often expensive, logistically difficult and environmentally sensitive. Numerical circulation models, based on a set of mathematical equations that govern fluid motion, provide a practical solution to the problem of coastal mixing.

In the water column, the behaviour of particles of organic matter is characterized by settling and deposition. Settling rate depends on the size, density and shape of particles. Stokes' law provides a basic framework for predicting the size dependence of particle settling. While discrete particles exhibit Stokes' settling behaviour, attractive forces also cause the formation of particle aggregates. Particle aggregation can effectively increase the settling flux of the original disaggregated particle mixture by repackaging small particles into larger, more rapidly sinking ones.

When particles settle from the water column, they may accumulate at the sediment-water interface or be subject to further transport and redistribution. When bottom shear stress exceeds a critical value, particles at the boundary between the sediment and water can be set in motion. Susceptibility to resuspension is set by a variety of sediment characteristics, including particle density and size, and degree of consolidation. Factors such as bioturbation have also been shown to effect erodibility of sediments (Andersen 2001). Numerical models are used to predict the temporal and spatial distribution of particulates in the marine environment.

ORGANIC ENRICHMENT AS A PROCESS

Organic matter flux of fish feces, waste feed and detached fouling organisms from cage surfaces, as well as natural particles, is monitored by measurements based on nitrogen or carbon in sedimentation traps. In the context of aquaculture, many authors examine carbon determined pyrolitically as a measurement of organic matter flux. However, the chemical and physical state of organic matter in sediments is not well understood. Thus, quantitative measures of total organic carbon are poor indicators of biochemical availability to biota.

The input of organic carbon to sediments, or sedimentation rate, is measured in suspended sediment traps. Reported sedimentation rates under and near salmon mariculture cages vary from 1 to 181 g $C{\cdot}m^{-2}{\cdot}day^{-1}$. *Notwithstanding differences in environmental conditions, farm size and fish stocking densities, some of the variation in sedimentation rates is due to inherent difficulties in suspended sediment trap methodology. The benthic input from fish feces and waste feed can also be measured by mass balance calculations: based on total organic carbon accumulated in sediment, total input of fish feed, growth period and surface area of the cage footprint. However, all farm sites have some water movement and therefore require an examination of where the particles are deposited. Cromey et al. (2002) developed a particle tracking model that uses depth and particle settling rates as affected by observed currents with modelled shear velocity and turbulence.*

In soft sediments receiving natural background levels of sedimentation (0.1 - 1.0 g C·m^{-2}·day^{-1}), the sediment-water interface is aerobic, and this aerobic layer contains abundant macrofauna and meiofauna. In contrast, soft depositional sediments receiving a continuous elevated level of sedimentation, for example due to fish feces and waste feed, become anoxic at the interface and develop a black, top-down sulfide layer. Some of the sulfide produced may also be oxidized by other chemoautotrophic bacteria (e.g. Beggiaotoa sp.) (Lumb and Fowler 1989). The white patches of this bacteria may be seen on the sediment surface under fish cages. Hargrave (1994) considered that flux rates of fish feces and waste feed of >1.0 g C·m^{-2}·day^{-1} caused marked organic enrichment effects in net depositional sediments under salmon cages. The process of organic enrichment in farm footprints requires a varying amount of time to reach an equilibrium point, depending on oceanographic and substrate conditions. Some studies have shown that organic enrichment can be reversed if the increased levels of sedimentation are stopped (Brooks et al. 2003).

The initial effect of a sudden addition of a high flux of readily decomposable organic carbon to sediments is increased metabolism by aerobic bacteria, leading to hypoxia and anoxia causing death of the most susceptible aerobic life forms (Gray et al. 2002). In the fish cage footprint, the bulk of the metabolism is by sulfate reduction at a higher rate than at reference stations (Holmer and Kristensen 1992) and results in the loss of nitrification and denitrification pathways (Kaspar et al. 1988). The death of burrowing macrofauna leads to a rapid decline in the capability for irrigation or aerated water within the upper profile and a more rapid development of anoxia. The predominant bacteria become anaerobes, principally sulfate reducers and methanogenic bacteria. Methane makes up the bulk of the outgassing from heavily impacted farm sediments (Wildish et al. 1990), and sulfate reduction produces hydrogen sulfide, which is readily oxidized in aerobic seawater.

Organic enrichment indices may be regarded as proxies of the ecosystem to indicate extent or amount of organic enrichment. The most widely accepted index is based on macrofaunal species, abundance and biomass. Organic enrichment gradients by Pearson and Rosenberg (1978) and Poole et al. (1978) can be arbitrarily divided into four groupings based on the species and density of macrofauna present. Both of these older indices suffer because the macrofaunal or microbial surveys required to determine them are expensive in time to obtain. Other methods can be linked to spatial or temporal versions of the organic enrichment gradients based on macrofauna. They include sediment profile imaging (Rhoads and Germano 1986; Nilsson and Rosenberg 1997) and sediment geochemistry by redox and sulfide measurements (Wildish et al. 2001).

Hargrave (1994) proposed a benthic enrichment index based on the product of organic carbon and redox. The concept is to determine the sedimentation rate from the carbon present in interfacial sediments. Cranston (1994) described a geochemical method that is a direct measure of net carbon burial rates. The method is based on downcore concentrations of sulfate and ammonium, as indicators of the remineralization rates, and requires large cores and numerous, expensive chemical determinations. However, the *method appears to be robust, and there is a positive linear relationship between sedimentation and carbon burial rates. Recently, Dell'Anno et al. (2002) suggested a suite of environmental variables to assess the organic enrichment status of the coastal zone in the Mediterranean sea, based on interfacial sedimentary variables.*

ECOLOGICAL EFFECTS OF ORGANIC ENRICHMENT

Numerous reviews of organic enrichment associated with many industries show two important generalities: the ecological response is complex, involving pelagic-benthic coupling and both water column and sediments; and the effects include all sources of organic matter, both natural and anthropogenic.

Different physical and biological conditions on the Atlantic and Pacific coasts of Canada result in differing ecological responses to aquaculture. In the Bay of Fundy, the salmon aquaculture industry does not usually experience severe hypoxia in seawater because of energetic tidal mixing. If hypoxia occurs, it is in sediment pore water and localized to the benthic footprint area. There are few available data on dissolved oxygen in the sediments and water column near fish farms in British Columbia. However, naturally occurring low (<4 $mg{\cdot}L^{-1}$) *dissolved oxygen levels are present on the west coast of Vancouver Island and in Queen Charlotte Strait during the late summer and early fall (Levings et al. 2002). On the Pacific coast, fish farms are located over deeper and more diverse seabed habitats and most tenures include a mosaic of sediment types and are not characterized by homogenous level mud bottoms. Rock terraces, cliff walls and boulder fields are also under fish farms in some locations (see Levings et al. 2002 and references therein).*

The general response of soft-sediment macrofaunal populations and communities to organic enrichment gradients is well established. It involves the local extinction of the resident equilibrium community, followed by the re-establishment of opportunists if conditions improve. Some species are more resistant to hypoxia than others (Diaz and Rosenberg 1995). In general, the crustaceans and echinoderms are most sensitive. Field studies suggest that, where seasonal hypoxia occurs, dissolved oxygen (DO) levels of 1 $ml{\cdot}L^{-1}$ *begin to cause macrofaunal invertebrate mortality (Diaz and Rosenberg 1995). In areas of permanent oxygen deficiency, the benthic communities appear to be adapted to an even lower critical DO level. Although very low DO levels are seen as the major limiting factor for macrofauna, the role of* H_2S *(and ammonia) is less clear. Where severe hypoxia is present, both may be released.*

Relatively little is known about the effects of organic enrichment on ecosystem functioning. Simplistic approaches to assess ecosystem effects assume that all of the secondary benthic production that becomes anoxic or hypoxic is lost to the next trophic level of predators. However, these approaches ignore that many individual predators are adapted to prey on a specific set of a few species in an equilibrium community.

Azoic or anoxic sediments could cause a significant shift in pelagic-benthic coupling. The effect of organic enrichment in sediments is to move the system to one dominated by *bacteria, ciliates and meiofauna, and where the trophic links to the next level of the food web are broken. In bays heavily occupied by salmon farms, Pohle et al. (2001) analyzed the macrofaunal community at reference stations and found significant structural change. While the cause could not be identified, the most probable explanation is that an aspect of enrichment linked to salmon farming caused changes in benthic-pelagic coupling in such a way as to exclude some species and encourage others. Although the severely hypoxic areas caused by fish farming in the Bay of Fundy are relatively small, no studies have examined benthic-pelagic coupling in the vicinity of fish farms.*

TOWARDS PREDICTIVE MODELS

Prediction of benthic organic enrichment from fish farms requires the application of mathematical models. Process-oriented models use mathematical frameworks that describe the major physical and biogeochemical components and the processes through which they interact. Ocean circulation models predict the transport and mixing in the coastal zone, including the dispersion of organic matter from fish farms. Comprehensive ocean models, such as the Princeton Ocean Model (Blumberg and Mellor 1987) and the CANDIE ocean model (Sheng et al. 1998), have been successfully applied to coastal waters. Sediment transport models predict the time evolution of the spatial distribution of suspended particulate matter, as well as the exchanges of material between the water column and the benthos. Diagenetic models couple water column processes to vertically resolved sediment biogeochemical models (Wijsman et al. 2000).

Empirical models use statistical descriptions of the relationships between observable quantities that are indicators for key environmental components or processes. Findlay and Watling (1997) proposed an oxygen-based framework, based on the balance between benthic oxygen supply to oxygen demand, for assessing the benthic response to organic enrichment from salmon aquaculture. Dudley et al. (2000) developed a more process-oriented approach using a transport model to estimate dispersion of wastes from a fish farm to the benthos. DEPOMOD uses a hybrid approach to model benthic enrichment effects, and includes a particle tracking model and empirical relationships between the spatial distribution of solids and changes in benthic community structure (Cromey et al. 2002). In British Columbia, Carswell and Chandler (2001) and Stucchi (in prep.) have developed particle tracking models that give estimates of size of the sediment field under and near fish farms, but these models are not yet linked with benthic biological data.

TOWARDS METHODS OF MONITORING ORGANIC ENRICHMENT

Several variables to assess organic enrichment in seawater have been identified, including phytoplankton species and abundance matrices, dissolved oxygen concentrations, and total nutrient concentrations. Because none of these variables is accepted as the single indicator of the trophic status of seawater, multiparameter classifications have been used. For example, the OECD classification is based on chlorophyll a, plant nutrients and Secchi depths (Vollenweider and Kerekes 1982).

There are several methods to assess organic impacts in sediments. Those methods based on classical macrofaunal sampling and analysis are among the best known, although most costly. Alternative methods, such as sediment profile imaging or geochemistry, are more cost-effective. Recently developed methods, such as aerial photography, video photography and multibeam acoustics, have the potential to produce detailed and accurate maps over large areas. However, these methods require further research and groundtruthing. For benthic monitoring, the presence of predominantly hard or soft substrates will dictate the type of sampling. Other considerations include size of the ecosystem and the primary goal of the study.

General types of monitoring to detect organic enrichment in the marine environment are distinguished by their purpose. Geographical studies determining the limit of impact benefit primarily from synoptic survey methods, such as remote sensing of chlorophyll a in surface waters, underwater photography, video photography and acoustic surveys of soft sediments. Studies involving site comparison (treatment/reference sites), temporal trends (before/after) and practical monitoring (relative impact) can use a few alternative methods. Sediment profile images and sediment geochemical methods provide a much more cost-effective and credible alternative to the use of macrofaunal sampling and analyses for routine monitoring purposes.

RESEARCH NEEDS

Research is needed to provide an understanding of processes and to provide input to models and monitoring programs. Research is also needed to aid the scientific assessment of organic enrichment from marine finfish aquaculture (or near-field enrichment of marine finfish aquaculture). The following specific research needs are identified:

- *Conduct sedimentology and physical and chemical oceanography studies, including coastal circulation, mixing, dispersion and transport processes in support of process models, and observation and modelling studies of water column particle dynamics.*
- *Conduct seasonal studies of organic enrichment (such as redox potential and sulfide) to examine ecological factors affecting organic enrichment events from salmon farming.*
- *Measure the availability of carbon to microbial decomposers.*
- *Determine effects of organic enrichment on coarse and hard substrates in British Columbia where fish farms are located over mosaics of sediment types.*
- *Determine effects of organic enrichment on ecosystem functioning to establish cause-effect relationships.*
- *Investigate how organic enrichment from aquaculture affects benthic-pelagic coupling.*
- *Undertake further fallowing studies in Pacific and Atlantic Canada.*
- *Verify models, such as Lagrangian-based particle models, sediment transport models and biogeochemical models, through collaborative efforts between modellers and field biologists.*

- *Predict holding capacity or assimilative limits related to the amount of local organic enrichment.*
- *Develop geographical survey methods, such as satellite, aerial surveillance, underwater video photography and acoustics.*
- *Devise new environmental monitoring methods.*
- *Calibrate, standardize and audit existing environmental monitoring methods.*

ENRICHISSEMENT ORGANIQUE À PROXIMITÉ DES INSTALLATIONS PISCICOLES EN MER

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RÉSUMÉ

Les auteurs analysent les ouvrages publiés sur l'enrichissement organique à proximité d'installations d'élevage intensif du poisson en mer. Les piscicultures constituent une source de matières organiques en suspension et dissoutes, provenant des excréments de poisson, des déchets d'aliments et des salissures détachées des cages. L'expression « à proximité » sert à distinguer les effets locaux (limités à la superficie des cages au fond) des effets à distance, et désigne les effets à l'intérieur de la superficie des sédiments couverte par les cages et entre le niveau des populations et de la communauté. Les effets de la mariculture à proximité sont restreints par les limites physiques de la dispersion et de la sédimentation des particules de déchets provenant de cages individuelles ou de fermes.

TRANSPORT, DISPERSION ET COMPORTEMENT DES PARTICULES

Pour étudier le devenir biogéochimique des matières organiques dans un écosystème benthique ou pélagique, il faut comprendre comment elles sont transportées et dispersées d'une pisciculture. Les matières organiques sont transportées des cages à poissons au milieu marin avoisinant par l'action de divers types de circulation de l'eau à proximité immédiate des cages. À mesure qu'elles se déplacent, elles se dispersent et leur teneur diminue, par dilution et par sédimentation.

Au Canada, les milieux côtiers où se pratique l'aquaculture sont caractérisés par des côtes accidentées et une topographie complexe. Par conséquent, ils présentent souvent des champs de courant hautement structurés et assez complexes. Dans les systèmes marins fortement stratifiés, les matières dissoutes peuvent être piégées de façon efficace dans les parties supérieure ou inférieure de la colonne d'eau. Dans le contexte de l'aquaculture, la stratification peut constituer un important facteur dans la dispersion des matières organiques provenant de certaines fermes situées à l'intérieur de fjords en Colombie-Britannique.

Des observations et la modélisation numérique permettent d'étudier le transport et la dispersion des particules dans les eaux côtières. Les études d'observation du mélange font souvent appel au mouillage de bouées dérivantes qui imite le mouvement de parcelles d'eau. Les expériences sur le terrain comportant le déversement de colorants dans l'océan permettent de mieux caractériser la dispersion à partir d'une source ponctuelle, mais elles sont souvent coûteuses et difficiles à faire sur le plan logistique et ne sont pas toujours acceptables d'un point de vue environnemental. Les modèles numériques de circulation, reposant sur un ensemble d'équations mathématiques décrivant le mouvement des fluides, constituent une solution pratique au problème du mélange côtier.

Dans la colonne d'eau, le comportement des particules de matières organiques est caractérisé par la sédimentation et le dépôt. La vitesse de sédimentation dépend de la taille, de la densité et de la forme des particules. La loi de Stoke fournit un cadre fondamental pour prédire la dépendance de la taille de la sédimentation des particules. Bien que les particules grenues montrent le comportement de sédimentation de Stoke, les forces d'attraction causent aussi la formation d'agrégats de particules. L'agrégation peut effectivement accroître le flux de sédimentation du mélange de particules non agrégées en agglomérant les petites particules en éléments plus gros, qui s'enfoncent plus rapidement.

Lorsque des particules sédimentent de la colonne d'eau, elles peuvent s'accumuler à l'interface sédiments-eau ou être transportées et redistribuées ailleurs. Lorsque la force de cisaillement au fond dépasse une valeur critique, les particules situées à l'interface entre les sédiments et l'eau peuvent être mises en mouvement. La susceptibilité à la remise en suspension est tributaire d'une gamme de caractéristiques des sédiments, y compris la densité et la taille des particules, ainsi que le degré de consolidation. Des facteurs tels la bioturbation ont aussi un effet sur l'érodabilité des sédiments (Andersen, 2001). Des modèles numériques sont utilisés pour prédire la distribution temporelle et spatiale de particules dans le milieu marin.

ENRICHISSEMENT ORGANIQUE COMME PROCESSUS

Des mesures de la teneur en azote ou en carbone dans des pièges à sédiments permettent de suivre le flux des matières organiques que constituent les excréments de poisson, les déchets d'aliments, les salissures détachées des cages et les particules d'origine naturelle. Dans le contexte de l'aquaculture, de nombreux auteurs considèrent la teneur en carbone établie pyrolitiquement comme mesure du flux des matières organiques. Mais comme l'état chimique et physique des matières organiques dans les sédiments est mal compris, les mesures quantitatives de la teneur en carbone organique total sont de mauvais indicateurs de sa disponibilité biochimique pour le biote.

L'apport de carbone organique aux sédiments, ou le taux de sédimentation, est mesuré à l'aide de pièges à sédiments suspendus. Les taux de sédimentation établis sous et près de cages à saumon varient entre 1 à 181 $g\ C\ m^{-2}\ d^{-1}$*. Indépendamment des différences dans* *les conditions environnementales, de la taille des fermes et des densités d'empoissonnement, une partie de la variation dans les taux de sédimentation est imputable à des problèmes inhérents à la méthode des pièges à sédiments suspendus. Des calculs du bilan massique, reposant sur le carbone organique total accumulé dans les sédiments, l'apport total d'aliments pour poissons, la période de croissance et la superficie au sol des cages, peuvent aussi servir à établir l'apport benthique provenant d'excréments de poisson et de déchets d'aliment. Mais, comme toutes les fermes sont soumises à un certain mouvement de l'eau, l'endroit où les particules sont déposées doit être établi. Cromey* et al. *(2002) ont élaboré un modèle de repérage des particules mettant en relation, d'une part, la profondeur et les taux de sédimentation des particules telles que modulées par les courants observés et, d'autre part, la vitesse de cisaillement et la turbulence modélisées.*

Dans les sédiments mous soumis à un apport sédimentaire naturel (à des taux allant de 0,1 à 1,0 g C m^{-2} d^{-1}*), l'interface sédiments-eau est aérobie; cette couche abrite une abondante macrofaune et méiofaune. Par contre, les sédiments de dépôt mous qui reçoivent un apport sédimentaire continu élevé, par exemple des excréments de poisson et des déchets d'aliments, deviennent anoxiques à l'interface et développent une couche sulfurée noire descendante. Une partie du sulfure produit peut aussi être oxydé par d'autres bactéries chimiotrophes (p. ex.,* Beggiaotoa *sp.) (Lumb et Fowler, 1989). Les couches blanches formées par cette bactérie sont visibles à la surface des sédiments sous les cages à poissons. Hargrave (1994) considère que des taux de flux des excréments de poisson et des déchets d'aliments de > 1,0 g C* m^{-2} d^{-1} *causent des effets d'enrichissement organique marqués dans les sédiments de dépôt nets sous les cages à saumon. Le temps pris par le processus d'enrichissement organique des superficies au sol des cages pour atteindre un point d'équilibre varie selon les conditions océanographiques et les conditions du substrat. Quelques études ont révélé que l'enrichissement organique peut être inversé si les niveaux accrus de sédimentation sont stoppés (Brooks* et al.*, 2003).*

L'ajout soudain d'un flux élevé de carbone organique facilement décomposable à des sédiments a comme effet initial d'augmenter la vitesse de métabolisme des bactéries aérobies, ce qui donne lieu à l'hypoxie et à l'anoxie causant la mort des formes vivantes aérobies les plus sensibles (Gray et al.*, 2002). Dans les superficies au sol des cages à poisson, la majorité du métabolisme se fait par réduction de sulfates à une vitesse plus élevée qu'aux stations de référence (Holmer et Kristensen, 1992) et résulte en la perte de voies de nitrification et de dénitrification (Kaspar* et al.*, 1988). La mort d'organismes macrofauniques fouisseurs mène à un déclin rapide de la capacité d'irrigation ou d'entrée d'eau aérée dans la partie supérieure de la colonne d'eau et au développement plus rapide de l'anoxie. Les bactéries prédominantes deviennent anaérobies, principalement des sulfatoréductrices et des méthanogènes. Le méthane constitue la majorité des gaz libérés des sédiments chargés gisant sous les fermes (Wildish* et al.*, 1990); la réduction de sulfates produit du sulfure d'hydrogène, qui est promptement oxydé dans l'eau de mer aérobie.*

Les indices d'enrichissement organique peuvent être considérés comme des indicateurs de l'écosystème pour indiquer l'ampleur ou le niveau d'enrichissement organique. L'indice le plus généralement accepté s'appuie sur les espèces macrofauniques, leur abondance et leur biomasse. Les gradients d'enrichissement organique de Pearson et Rosenberg (1978) et de Poole et al. *(1978) peuvent être arbitrairement divisés en quatre groupes fondés sur les espèces macrofauniques présentes et leur densité. Mais ces deux anciens indices souffrent du fait que les relevés de la macrofaune ou des microbes requis pour les établir sont chronophages. D'autres méthodes peuvent être enchaînées aux versions spatiales ou temporelles des gradients d'enrichissement organique reposant sur la macrofaune. Celles-ci incluent l'imagerie des profils sédimentaires (Rhoads et Germano, 1986; Nilsson et Rosenberg, 1997) et la géochimie des sédiments par mesure de l'oxydoréduction et des sulfures (Wildish* et al.*, 2001).*

Hargrave (1994) a proposé un indice d'enrichissement benthique reposant sur l'oxydoréduction du carbone organique, le concept étant d'établir le taux de sédimentation à partir du carbone présent dans les sédiments de surface. Cranston (1994) a décrit une méthode géochimique, une mesure directe des taux d'enfouissement nets du carbone. Cette méthode repose sur les teneurs descendantes en sulfates et en ammonium dans les carottes, à titre d'indicateurs des taux de reminéralisation, et requiert de grosses carottes et de nombreuses analyses chimiques dispendieuses. Elle semble toutefois robuste, et il existe une relation linéaire positive entre le taux de sédimentation et le taux d'enfouissement du carbone. Récemment, Dell'Anno et al. (2002) ont proposé une série de variables environnementales pour évaluer l'état d'enrichissement organique de la zone côtière de la Méditerranée, reposant sur des variables sédimentaires interfaciales.

EFFETS ÉCOLOGIQUES DE L'ENRICHISSEMENT ORGANIQUE

De nombreux examens de l'enrichissement organique associé à diverses industries révèlent deux caractéristiques générales : la réponse écologique est complexe, mettant en jeu un couplage pélagique-benthique ainsi que la colonne d'eau et les sédiments; et les effets incluent toutes les sources de matières organiques, tant naturelles qu'anthropiques.

Les conditions physiques et biologiques étant différentes sur les côtes canadiennes de l'Atlantique et du Pacifique, les réponses écologiques à l'aquaculture diffèrent. Dans la baie de Fundy, l'industrie de la salmoniculture en mer ne connaît habituellement pas une hypoxie grave à cause du vigoureux mélange tidal. Si l'hypoxie se produit, elle se manifestera dans l'eau interstitielle des sédiments et sera restreinte à la superficie benthique des cages. Peu de données sont disponibles sur les teneurs en oxygène dissous dans les sédiments et la colonne d'eau près des piscicultures en Colombie-Britannique. Toutefois, la côte Ouest de l'île de Vancouver et le détroit de la Reine-Charlotte connaissent de faibles teneurs en oxygène dissous (< 4 mg L^{-1}) d'origine naturelle à la fin de l'été et au début de l'automne (Levings et al.*, 2002). Sur la côte du Pacifique, les piscicultures sont établies au-dessus de parcelles d'habitat benthique plus profondes et plus diversifiées et la plupart des concessions incluent une mosaïque de types de* *sédiment; le niveau des fonds vaseux y est en outre variable. Des terrasses rocheuses, des falaises et des champs de blocs gisent aussi sous les fermes à certains endroits (voir Levings* et al., *2002 et les références qui y sont citées).*

La réponse générale des populations et des communautés macrofauniques des sédiments mous aux gradients d'enrichissement organique est bien établie. Elle comprend la disparition locale de la communauté résidente d'équilibre, suivie du réétablissement des espèces opportunistes si les conditions s'améliorent. Certaines espèces sont plus résistantes que d'autres à l'hypoxie (Diaz et Rosenberg, 1995). En général, les crustacés et les échinodermes sont les plus sensibles. Les résultats d'études sur le terrain donnent à penser que, lorsqu'une hypoxie saisonnière se produit, des teneurs en oxygène dissous (O.D.) de 1 ml L^{-1} *commencent à causer la mort des invertébrés macrofauniques (Diaz et Rosenberg, 1995). Aux endroits où les teneurs en oxygène sont faibles en permanence, les communautés benthiques semblent être adaptées à une teneur critique en O.D. même plus faible. Quoique des teneurs en O.D. très faibles soient considérées comme le principal facteur limitant pour la macrofaune, le rôle de* H_2S *(et de l'ammoniac) est moins clair. Lorsqu'une hypoxie grave se manifeste, ces deux gaz peuvent être libérés.*

On sait relativement peu au sujet des effets de l'enrichissement organique sur la dynamique des écosystèmes. Les méthodes simples d'évaluation de ces effets supposent que toute la production benthique secondaire qui devient anoxique ou hypoxique est avalée par le niveau trophique suivant de prédateurs. Mais ces méthodes ne tiennent pas compte du fait que de nombreux prédateurs sont adaptés à chasser un ensemble particulier de quelques espèces dans une communauté à l'équilibre.

Les sédiments azoïques ou anoxiques pourraient causer un important déplacement dans le couplage pélagique-benthique. L'effet de l'enrichissement organique des sédiments est de transformer un système en un autre dominé par des bactéries, des ciliés et des organismes méiofauniques, et où les liens trophiques au niveau suivant de la chaîne alimentaire sont brisés. Pohle et al. *(2001) ont analysé la communauté macrofaunique à des stations de référence dans des baies où les salmonicultures étaient trés proches les unes des autres et ont découvert d'importants changements structurels. Bien qu'ils n'aient pu en identifier la cause, l'explication la plus probable est qu'un aspect de l'enrichissement lié à la salmoniculture a causé des changements dans le couplage benthique-pélagique de sorte que certaines espèces en ont été exclues et que d'autres ont été favorisées. Bien que les secteurs gravement hypoxiques résultant de la pisciculture dans la baie de Fundy soient relativement petits, aucune étude du couplage benthique-pélagique dans le voisinage des fermes n'a été faite.*

VERS DES MODÈLES DE PRÉVISION

La prédiction de l'enrichissement organique de la zone benthique imputable à des piscicultures requiert l'application de modèles mathématiques. Les modèles axés sur les processus utilisent des cadres mathématiques qui décrivent les principaux éléments physiques et biogéochimiques et les processus par lesquels ils interagisssent. Les modèles *de circulation océanique permettent de prédire le transport et le mélange dans la zone côtière, y compris la dispersion des matières organiques provenant des piscicultures. Les modèles polyvalents des océans, comme le modèle Princeton (Blumberg et Mellor, 1987) et le modèle CANDIE (Sheng* et al., *1998), ont été appliqués avec succès aux eaux côtières. Les modèles de transport des sédiments permettent de prédire l'évolution dans le temps de la distribution spatiale des particules en suspension, ainsi que les échanges de matières entre la colonne d'eau et le benthos. Les modèles diagénétiques couplent les processus de la colonne d'eau avec les modèles résolus verticalement de la biogéochimie des sédiments (Wijsman* et al., *2000).*

Les modèles empiriques utilisent des descriptions statistiques des relations entre les facteurs quantitatifs observables indicateurs d'éléments ou de processus environnementaux clés. Findlay et Watling (1997) ont proposé un cadre axé sur le ratio de l'oxygène, reposant sur l'équilibre entre la disponibilité et la demande en oxygène dans la zone benthique, pour évaluer la réponse au niveau benthique à l'enrichissement organique imputable à la salmoniculture. Dudley et al. *(2000) ont élaboré une démarche davantage orientée sur les processus faisant appel à un modèle de transport pour estimer la dispersion des déchets provenant d'une pisciculture dans le benthos. DEPOMOD fait appel à une démarche hybride pour modéliser les effets d'enrichissement benthique et inclut un modèle de dépistage des particules et des relations empiriques entre la distribution spatiale des solides et des changements dans la structure de la communauté benthique (Cromey* et al., *2002). Pour la Colombie-Britannique, Carswell et Chandler (2001) et Stucchi (2005) ont élaboré des modèles de dépistage des particules qui donnent des estimations de la superficie du champ de sédiments sous et près de fermes, mais un lien n'a pas encore été établi entre ces modèles et des données biologiques sur le benthos.*

VERS DES MÉTHODES DE SURVEILLANCE DE L'ENRICHISSEMENT ORGANIQUE

Plusieurs variables pour évaluer l'enrichissement organique en mer ont été identifiées, y compris des espèces phytoplanctoniques et des matrices de leur abondance, les teneurs en oxygène dissous et les concentrations totales en substances nutritives. Comme aucune de ces variables n'est reconnue comme l'unique indicateur de l'état trophique de la mer, des classifications à paramètres multiples ont été utilisées. Par exemple, la classification de l'OCDE repose sur la teneur en chlorophylle a, les concentrations d'éléments nutritifs pour les végétaux et les profondeurs d'après le disque de Secchi (Vollenweider et Kerekes, 1982).

Il existe plusieurs méthodes pour évaluer les effets des matières organiques dans les sédiments. Celles-ci, qui reposent sur l'échantillonnage et l'analyse conventionnelles de la macrofaune, s'inscrivent parmi les mieux connues, quoique la plupart soient dispendieuses. D'autres, comme l'imagerie des profils sédimentaires ou la géochimie, sont plus économiques. Des méthodes récemment mises au point, comme la photographie aérienne, la photographie vidéo et l'acoustique à faisceaux multiples, offrent le potentiel de produire des cartes détaillées et précises de grandes régions. Ces méthodes doivent *toutefois faire l'objet d'autres recherches et de vérifications sur place. Pour ce qui est de la surveillance du milieu benthique, la présence de substrats principalement durs ou mous déterminera le type d'échantillonnage. La superficie de l'écosystème et le but primaire de l'étude sont d'autres considérations.*

Les types généraux de méthodes de surveillance utilisées pour déceler l'enrichissement organique dans le milieu marin se distinguent par leur but. Les études géographiques visant à déterminer la limite des effets s'appuient principalement sur des méthodes de relevé synoptique, comme la télédétection de la teneur en chlorophylle a dans les eaux de surface, la photographie sous-marine, la photographie vidéo et des relevés acoustiques des sédiments mous. Les études portant sur la comparaison de sites (sites traités/sites de référence), les tendances temporelles (avant/après) et la surveillance pratique (effets relatifs) peuvent faire appel à d'autres méthodes. Les images des profils sédimentaires et les méthodes de détermination de la géochimie des sédiments sont une solution beaucoup plus économique et aussi crédible que l'échantillonnage et les analyses de la macrofaune aux fins de surveillance routinière.

BESOINS EN RECHERCHES

Des recherches sont nécessaires pour mieux comprendre les processus et obtenir des données d'entrée pour les modèles et les programmes de surveillance. Des recherches sont nécessaires aussi aux fins d'évaluation scientifique de l'enrichissement organique imputable à la pisciculture en mer (ou l'enrichissement à promité des installations piscicoles en mer). Voici les besoins en recherches particuliers :

- *Il faut mener des études de la sédimentologie et de l'océanographie physique et chimique, y compris les processus de circulation côtière, de mélange, de dispersion et de transport, afin d'étayer les modèles des processus, et des études d'observation et de modélisation de la dynamique des particules dans la colonne d'eau.*
- *Il faut mener des études saisonnières de l'enrichissement organique (comme le potentiel d'oxydoréduction et les teneurs en sulfure) en vue d'identifier les facteurs écologiques ayant une incidence sur les épisodes d'enrichissement organique imputable à la salmoniculture.*
- *Il faut mesurer la disponibilité du carbone pour les micro-organismes décomposeurs.*
- *Il faut déterminer les effets de l'enrichissement organique sur les substrats grossiers et durs en Colombie-Britannique où des piscicultures sont établies sur des mosaïques de types de sédiments.*
- *Il faut déterminer les effets de l'enrichissement organique sur la dynamique des écosystèmes en vue d'établir les relations de cause à effet.*
- *Il faut examiner dans quelle mesure l'enrichissement organique imputable à l'aquaculture a un effet sur le couplage benthique-pélagique.*
- *Il faut mener de nouvelles études sur la mise en jachère sur les côtes canadiennes de l'Atlantique et du Pacifique.*
- *Il faut vérifier des divers modèles, comme les modèles Lagrangien de dispersion des particules, des modèles de transport des sédiments, et des modèles biogéochimiques, par le biais d'efforts concertés des modélisateurs et des biologistes sur le terrain.*

- *Il faut prédire la capacité de captage ou les limites d'autoépuration des sédiments reliées au niveau d'enrichissement organique local.*
- *Il faut élaborer des méthodes de relevé géographique, par satellite, par surveillance aérienne, par photographie vidéo sous-marine et par acoustique.*
- *Il faut concevoir de nouvelles méthodes de surveillance environnementale.*
- *Il faut étalonner, normaliser et vérifier les méthodes existantes de surveillance environnementale.*

INTRODUCTION

The pragmatic use of the terms near-field (this paper) and far-field (Hargrave 2003) arose from the realization that organic enrichment effects at salmon farms could be local (footprint limited) or more distant (far-field) following seawater transport, hypernutrification and eutrophication. An example of far-field organic enrichment is the increased growth of *Ulva* and *Enteromorpha* (green macroalgae) on intertidal clam flats distant from salmon farms in the Bay of Fundy (Auffrey et al. 2003). Our use of the term near-field implies that our focus will be on the sedimentary footprint and thus, in ecological terms, between the population and community level. Our concept of the near-field in relation to mariculture is bounded by the physical limits of particulate waste dispersal and sedimentation from individual cages or farms (Fig. 1). Organic wastes from finfish aquaculture originate as fish feces, uneaten feed and net-cleaning wastes, which are then dispersed by tidal and wind/wave transport.

As a process, organic enrichment may be driven by natural (e.g. a dead whale reaching the seafloor) or anthropogenic sources (e.g. municipal or industrial wastes, fish processing, agriculture, as well as aquaculture). The enrichment process may occur in the water column and/or sediment as it becomes an integral part of pelagic-benthic coupling (Fig. 2). The partial carbon budget shown emphasizes: the links between pelagos and benthos, how settling particulate organic matter drives sediment metabolism and secondary benthic production, the interaction of all processes with physical forcing factors, notably temperature and water movement.

Near-field effects of organic enrichment resulting from finfish aquaculture are important because in a net depositional environment, they are fast acting and, initially, the only one indicating any (sedimentary) impact. For these reasons, near-field effects are the ones most frequently used in environmental monitoring. For erosional sediments, fish farm wastes may cause little or no near-field effects, although they contribute to far-field effects elsewhere. Far-field effects take longer to develop and are more difficult to detect (Hargrave 2003). Because of the links between benthos and pelagos outlined above, it is clear that a comprehensive ecological research or monitoring program needs to include both near- and far-field effects. Thus, the distinction between near- and far-field made herein is clearly an arbitrary one.

The aim of this overview is to assess the literature on near-field organic enrichment resulting from intensive marine finfish aquaculture. It is not intended to be an exhaustive review of the World literature on this subject. The emphasis is on Canadian finfish mariculture on the Atlantic and Pacific coasts, and an attempt is made to outline the further environmental research needed in these regions, particularly predictive modelling and monitoring of organic enrichment. Peer-reviewed publications began appearing on effects of organic enrichment from finfish aquaculture on the Atlantic coast of Canada (specifically the Bay of Fundy) in 1988, but the first papers began appearing from the Pacific coast in 2001. Therefore the vast majority of the information reviewed is from the Atlantic region, where we found 17 publications compared to five (5) from the Pacific.

MARINE FINFISH CULTURE HABITATS IN CANADA

SALMONID PRODUCTION STATISTICS

The total Canadian salmonid mariculture production in 2001 amounted to 110,000 tonnes (DFO Statistical Services, http://www.dfo-mpo.gc.ca/communic/statistics/aquacult/Aqua_E.htm). Of this total, British Columbia produced 62% (both Atlantic and Pacific salmon), New Brunswick produced 31% (Atlantic salmon), Nova Scotia produced 5% (Atlantic salmon and steelheads), and Newfoundland produced 2% (Atlantic salmon and steelheads).

Historically, the growth of salmonid mariculture in Canada (Table 1) suggests a rapid growth spurt after 1987. The early start in British Columbia was with Pacific salmon (chinook and coho), but not until Atlantic salmon were cultured did the industry in Canada begin its rapid expansion phase.

SITES OF PRODUCTION

Salmon farms on the Pacific coast are currently concentrated on Vancouver Island and on the south-central coast of mainland British Columbia (Fig. 3), but there also a few farms on the north coast. Most of the farms are located in coastal seaways, embayments or the seaward areas of the fjords. Because of the steep topography of the Pacific coast, the farms are generally located over water at least 30 m deep. Farms are generally not located in the inner parts of the fjords because the reduced salinity conditions there are not conducive to salmon culture. There are also farms in Puget Sound, Washington, south of the Strait of Georgia.

Salmon farms on the Atlantic coast (Fig. 4) are found in the United States (Maine), New Brunswick, Nova Scotia and Newfoundland. The largest concentration of farms, 97 in 2001, is in the Bay of Fundy. This industry is limited, with current cage technology, to wind/wave-protected coves and embayments and locations where winter ice is not a problem (Page and Robinson 1992). The possibility of winter ice and direct mortalities of salmon due to sub-zero seawater temperatures increases at inshore and estuarine locations. In some years, considerable losses due to winter chill can occur (Page and Robinson 1992).

PACIFIC/ATLANTIC COAST FINFISH CULTURE HABITATS

Pacific coast farms in British Columbia (BC) are located between the latitudes 48-51°N and experience a warm/temperate climate. Poor environmental conditions for siting salmon farms in the province (Caine 1987, cited in Levings 1994) are considered to be those where the summer seawater temperatures exceed 21°C and winter temperatures drop below 4°C. The tidal range was 4-5 m. The average depth at low water for 11 farms measured in 2001 (n = 53) was 48.2 m, with a range of 20-88 m. In June 2003, there were

125 marine salmon farms sites in BC with Crown Land Tenures and current Provincial aquaculture licences, with additional farms undergoing fallowing or being re-located.

In Atlantic Canada, the Bay of Fundy salmon culture industry is concentrated within wind/wave-protected bays in the Fundy Isles area. The average depth at low water for 93 active Bay of Fundy farms in 2001 was 14.9 m with a range of 5-40 m (Wildish et al. 2003). The climate is cold/temperate with the industry centered around 45°N. The Fundy Isles at the mouth of the Bay of Fundy has high tidal ranges of 8-12 m and, hence, usually with energetic tidal currents capable of strong flushing action.

Both the Pacific and Atlantic coastlines are complex and thus the site characteristics, dependent on currents and wave exposure, will vary in a very site-specific way.

PHYSICAL TRANSPORT AND DISPERSION OF ORGANIC MATTER

This paper considers the fate of organic matter produced by marine finfish culture, and our focus is on the accumulation of this material on the sediment in the process of organic enrichment. Prior to any consideration of this process, we must address the following question: *how is material transported away from its generating site and into the marine environment, and how does it make its way to the sediment?* This section discusses the physical processes which act to define the near-field effects of finfish aquaculture, or the so-called benthic "footprint" of a fish farm. This sets the stage for our examination of the near-field effects of organic enrichment. The depiction in Fig. 1 provides an illustration of the processes involved in organic matter deposition from cage-based finfish aquaculture. An additional process, not shown in Fig. 1, is wind/wave resuspension of surficial sediments.

Fish farms are a source of suspended and dissolved organic matter. This material is generated within fish cages as an unavoidable byproduct of culture activities. It consists of excess fish feed, fecal matter and other excretion products, as well as chemicals from pesticide treatments (see Burridge 2003). At a fundamental level, it is necessary to understand how this material is transported and dispersed from the fish farm into the marine environment. Only then can the consideration of its biogeochemical fate in the benthic or pelagic ecosystem be properly addressed.

Transport of material from a fish farm into the surrounding marine environment can be treated from the perspective of dispersal from a point source, which is a classic problem in environmental fluid dynamics. The idea behind this may be explained as follows. Suppose that at a given instant in time, a substance is only found at a single location, with zero concentration elsewhere. As the substance is released from this point, it spreads out and is diluted by the water motion. If the substance continues to be added over time, this is called a continuous point source release. This class of problem is the basis for addressing common environmental engineering problems, such as the release of material from a smokestack into the atmosphere or from a sewage outfall into the ocean. It has been considered in some detail in the excellent texts by Csanady (1973) and Fischer et al.

(1979). It also provides the basis for considering the near-field effects of organic matter produced by marine finfish culture. Organic matter is produced in a fish cage (or cages) and, in the context of the surrounding environment, can be idealized as a point source (or as a collection of point sources constituting a distributed source) that is continuously releasing material into the marine environment.

Two main elements of this continuous point source dispersal are relevant to the fate of organic matter from marine finfish culture: (1) *Transport and dispersal* and (2) *Particle behaviour*. Transport describes the movement of dissolved or suspended material by water currents. Dispersal is brought about by turbulent diffusion which acts to spread and dilute these particles due to mixing processes. Particle behaviour includes all aspects of particle motion which cause the particle trajectory to deviate from that of the fluid. This will include the settling of matter from the water column to the benthos and, where current conditions permit, the entrainment or resuspension of benthic organic material and its reincorporation into the water column.

TRANSPORT AND DISPERSAL

The movement and distribution of dissolved and suspended substances in ocean waters is governed by fluid motion. These processes are often collectively referred to as mixing, which may be defined as any process that causes a parcel of water to be mingled with, or diluted by, another. Here, a parcel of water refers to a small volume element of ocean water, which we often, and somewhat inaccurately, refer to as a particle. Coastal ocean mixing is driven by the physical processes of (i) *transport* (which moves water parcels about with the prevailing currents) and (ii) *dispersion* (which allows water parcels in close proximity to blend their material properties with one another). Transport provides for large-scale movement and defines pathways, while dispersion results in the dilution of material water properties. From the perspective of marine finfish culture, organic matter produced in fish cages is transported to the surrounding marine environment by the action of water currents in the immediate vicinity of the cages. As this material moves, it spreads out, with its concentration decreasing due to the action of incoherent small-scale water motions or turbulent diffusion.

Coastal environments where aquaculture occurs in Canada are characterized by irregular coastlines and complex topography. As a result, they often exhibit highly structured and quite complex flow fields. Horizontal mixing processes in coastal waters, such as those induced by tidal flows, are strongly dependent on the process of shear dispersion (Taylor 1953, 1954; Okubo 1968), the major result being greatly enhanced mixing, or dispersion that can occur in flows that are spatially variable or sheared. Okubo (1971) demonstrated the dependence of dispersion on spatial scale, and his mixing diagram remains the standard for many practical applications of coastal mixing problems. Another milestone in understanding coastal mixing was motivated by the observation that drifting buoys released in the ocean take a variety of unique pathways. This is indicative of the complex patterns of mixing of material substances for realistic coastal flow fields. Zimmermann (1986) first systematically studied this phenomenon and termed this type of mixing

"Lagrangian chaos." It has lead to the concept of anomalous diffusion (Jones 1991) and a recognition that very complex mixing regimes can arise from quite simple periodic flows (Aref 1984).

An important element which influences the vertical mixing of material properties is stratification, or changes in seawater density with depth. Density of seawater depends mainly on temperature and salinity. Stratification arises due to a lighter layer of water overlying more dense water. The interface between these two layers, the pycnocline, will tend to act as a barrier to the vertical mixing of material water properties.

Approaches to studying transport and dispersion in the coastal zone include observations, theory and numerical modelling. Observational studies of mixing in the coastal zone often rely on the deployment of drifting buoys. Modern drifters are designed to mimic the movement of water parcels. They are released from the location of interest and automatically record their positions at preset time intervals as they move with the ocean waters. The resulting data can give an idea of the pathways of organic matter transport from a fish farm. Unfortunately, a very large number of drifters is needed to build up the information required for a complete characterization of dispersion processes (Thompson et al. 2002). Field experiments that release coloured dye into the ocean are better able to characterize dispersion from a point source, but these are often expensive, logistically difficult and environmentally sensitive. They are generally used only for targeted scientific studies which are focused on validating theoretical predictions of mixing (Sundermeyer and Ledwell 2001). Measurements of small-scale microstructure in the temperature or velocity fields also provide fundamental information on ocean mixing processes (Lozovatsky and Fernando 2002).

Many features of coastal mixing can be derived from analytical solutions to simple mixing scenarios, as well as from scale analysis of more complex ones (Csanady 1973; Fischer et al. 1979). These results are often quite accurate and robust if carefully applied by experts with an explicit recognition of when they should be applied, and of the limitations under which they were derived. For many studies, these estimates of the bulk properties of mixing are entirely adequate.

For more complex situations, numerical models, based on a set of mathematical equations that govern transport and dispersion, provide a solution to the mixing problem. These are implemented on a computer and readily take into account site-specific features (discussed further on p. 32). The temporal and spatial evolution of dissolved material is predicted by solving tracer equations (Huthnance et al. 1993). A less common, but perhaps ultimately more satisfactory, approach is based on Lagrangian methods. Such a representation of mixing relies on a framework which follows the motion of the particle, rather than using a coordinate system at fixed points in space, as do the tracer equations. Stochastic Lagrangian approaches have been used to study mixing in atmospheric boundary layers (Rodean 1996) and are beginning to be applied to the ocean (Brickman and Smith 2002). Practical applications of these approaches are computationally costly and rely on Monte Carlo simulation techniques. However, simplified methods have been developed to synthesize bulk mixing properties from such simulations (Stull 1993; Thompson et al. 2002).

PARTICLE BEHAVIOUR

The elements of transport and dispersal outlined above focused on particles whose trajectories exactly match that of fluid elements. Organic matter from fish farms is comprised of particles with a variety of sizes and shapes, many of which will have a density that is different from seawater. Such particles will tend to deviate from the pathways of water motions, for example, by sinking. Below we consider some aspects of particle behaviour for both the water column and the benthos.

An important physical process which characterizes particle behaviour in the water column is settling and deposition. Settling rate has a functional dependence on the size and density of particles and, to a lesser extent, their shape. Stokes' law provides a basic framework for predicting the size dependence of particle settling. It computes the net force balance on a particle as the difference between buoyancy forces and drag forces. For a given density, large particles settle more rapidly than small particles. Discrete particles exhibit Stokes' settling behaviour, but attractive forces also cause the formation of particle aggregates, also known as flocs. The process of aggregation (or flocculation) occurs because of electrostatic forces and stickiness due to organic coatings that act together to bind particles. The level of water turbulence is also important. Turbulence may facilitate aggregation by bringing particles together, but at higher energies, disaggregation occurs due to shear stresses imposed by turbulent motions (Winterwerp 1998). Particle aggregation can effectively increase the settling flux of the original disaggregated particle mixture by repackaging small particles into larger, more rapidly sinking ones. It also alters the biological, physical and chemical properties of the particle field (Droppo 2001).

Seabed morphology factors influencing near-bed horizontal transport of fish farm wastes in the benthic boundary or nepheloid layer found near the bottom of the seabed should also be considered. On the Pacific coast, the steep and irregular underwater topography can lead to slumping of waste material and buildup of organic material in pockets or depressions in areas that superficially might be considered erosional. In addition, unlike the situation in New Brunswick, fish farm tenures in BC usually include a mosaic of sediment types ranging from mud to shell hash to boulder fields. Each of these sediment types have a different influence on particles moving in water near the seafloor.

When particles sink from the water column, they may accumulate at the sediment-water interface or be subject to further transport and redistribution. For example, Uncles et al. (1996) studied the movement of near fluidized mud beds in an estuarine environment where particles alternately settled out, only to be resuspended again and transported. The dynamics of the benthic boundary layer, along with the particle character, determine whether depositional or erosional processes will dominate. A moving fluid acts against a solid bottom boundary to produce shear stresses. The particles which form the boundary between the sediment and the water can be set in motion once the bottom shear stress exceeds a critical value. Bedload transport occurs when particles undergo movement but still remain confined to the zone immediately adjacent to the seabed. With increased shear stress, particles will be entrained into the moving fluid as suspended particulate matter and are transported as suspended load. Clearly, interaction between the water column and the sediment depends strongly on the physical properties of the sediment-water interface. Susceptibility to resuspension is set by a variety of sediment characteristics: for example particle density and size, as well as its degree of consolidation. Factors such as bioturbation have also been shown to effect erodibility of sediments (Andersen 2001).

Realistic predictions of the temporal and spatial distribution of sediment in the marine environment rely on numerical models. These are generalizations of the tracer models discussed earlier and include representations of the many physical processes governing sediment dynamics. In addition, they must also include detailed computations of the hydrodynamics and turbulence fields. A wide range of time scales is important: from benthic boundary layers in the short term, to residual circulation in the long term (Prandle et al. 1993). Such sediment dynamic models are well developed for scientific and engineering applications. These have a sound theoretical basis, but include a great many nonlinear processes and require a large number of parameters to be specified. Such models are discussed in the section on predictive models (p. 32).

In shallow coastal ecosystems, there is a strong two-way coupling between the water column and the benthos (Heip et al. 1995; Soetaert et al. 2000). This is driven by the input of organic matter which settles to the seafloor. The biological and chemical makeup of these particles may be quite variable (Engel et al. 2002). Pore water containing particulate organic matter deposited on the seafloor is transported into the sediment matrix through advective and diffusive processes. This detrital organic matter then undergoes a sequence of biogeochemical reactions whereupon remineralized, inorganic products are released as a diffusive flux into the water column. Diagenetic models are vertically resolved sediment biogeochemical models concerned with a detailed accounting of these processes (Boudreau 1997). A small fraction of this input flux will enter the refractory part of the benthic organic matter pool, thereby escaping mineralization and being permanently removed into the sediment (Middleburg et al. 1997). Models of these processes are discussed further in the section on predictive models (p. 32).

ORGANIC ENRICHMENT AS A PROCESS

Organic matter flux of fish feces, waste feed and detached fouling organisms from cage surfaces, as well as natural particles, is monitored by measurements based on nitrogen or carbon in sedimentation traps. In the context of aquaculture ecology, most authors have chosen some form of organic matter determination, inclusive of total volatile solids or total organic carbon, as the monitored species. Quantitative measures of total organic carbon, however, do not indicate the biochemical availability of organic matter to the benthic community. We mean by this that organic matter determinations are poor indicators of biodegradability: for example, wood fibers are high in enzyme resistant lignins versus salmon feces which are high in amino acids and other readily available nutrients for microbes.

CARBON LOADING OF SEDIMENTS

The input of organic carbon to sediments occurs at a sedimentation rate which can be measured by suspended sediment traps. After collection of suspended organic matter on filters, the dry weight is determined (as organic matter), followed by pyrolytic determination of its organic carbon. Results with non-standardized methods (Table 2) vary by >180 times. Leaving aside obvious differences in farm size and fish stocking densities, the variation is due to inherent difficulties in suspended sediment trap methodology. These include the following:

- where to site and at what depth in the water column to place the traps;
- biased sampling by traps. Butman (1986) has shown that sampling efficiency varies with current velocity. The aspect ratio of trap design needs to be adjusted for local water movement patterns, although with an aspect ratio of >5-1, should be suitable for most conditions;
- near-bottom traps may not have been corrected for resuspension which is caused by irregular wind/wave events;
- insufficient trap representation in both space and time. This is because sedimentation from salmon cages is a stochastic event. Salmon cages are square (30 x 30 m) or circular (50-70 m diameter), and the waste organic matter (waste feed, fecal matter, scales) produced originates in the area circumscribed by the cage. Using sediment trap data, dispersal of the organic material can occur at least 120 m from the edge of the cage, as shown by a study in BC (Brooks and Mahnken 2003). The one or few traps (up to six) deployed per cage may not adequately sample the wastes generated, as chemical and biological effects of the waste were reported up to between 145-185 m and 205-225 m, respectively (Brooks et al. 2003) from the edge of the cage. Sediment trap deployment times reported in Table 1 vary widely: from 40 min (Sutherland et al. 2001) to 30 d (Findlay and Watling 1997; Brooks 2001). In the latter case, aerobic metabolism within the trap may occur if a fixative was not present during deployment. Resuspension events have been studied by Dudley et al. (2000) using an *in situ* annular flume; and
- accounting for input of terrigenous carbon from adjacent land, particularly in BC where rivers are a significant source of carbon for coastal waters (Johannessen et al. 2003).

Another way of measuring the benthic input from fish feces and waste feed is by mass balance considerations. As an example, we detail an estimate for a salmon cage of 50-m diameter with a final harvest of 7000 salmon of 5-kg live weight. A 5-kg salmon utilizes 6.9 kg of moisture-free feed from smolt to harvest (Peterson et al. 2001), for a total input of 48.3 tonnes of feed per cage. Assume also that the sediment accumulates only 23% of the total organic carbon supplied as feed (Ackefors and Enell 1994), then:

- sedimentation total input is: 23% x 48.3 t ÷ (610 d x 2) = 9105.74 $g \cdot d^{-1}$ average daily input per cage over a 20-mo (= 610 d) growth period and assuming that only half of the feed is organic carbon;
- the surface area of the depositional area is the same as the cage area: πr^2 where r = 25 m = 1962.5 m^2;
- the mean daily input per unit area is: 9106/1962.5 = 4.64 $g\ C \cdot m^{-2} \cdot d^{-1}$, assuming that all the fish cage materials settle only in the immediate cage area; and for increasing fish numbers as is shown in Table 3.

For a site with minimum water movement and depositional sediments, this estimate might be of the same order of magnitude. All farm sites would have at least some water movement as shown in the footprint diagram of Fig. 1. In these cases, it would be required to determine where the particles deposit. This in turn would require detailed study of the water movement patterns at each site. Cromey et al. (2002) have developed a particle tracking model that uses depth and particle settling rates as affected by observed currents with modelled shear velocity and turbulence. They report that the model and observed results (shown in Table 2) agree within 20%. Rosenthal et al. (1988) estimated the total organic carbon inputs, as biological oxygen demand (BOD) loads, from a Danish trout farm as soluble, 2.6 kg BOD t^{-1} of finished trout per day, and particulate, 6 kg BOD $t^{-1} \cdot d^{-1}$. The above calculations do not account for the pronounced seasonality which occurs in salmon feeding rates of the Atlantic coast (Peterson et al. 2001).

BIOCHEMICAL AVAILABILITY OF CARBON

The chemical and physical state of organic matter in sediments is poorly understood, although it can be divided into biopolymers that are relatively labile and geopolymers that are resistant to microbial processing (Mayer 1989). Thus, measures of carbon made in the marine environment do not always provide good estimates of their biochemical availability to biota and, hence, are not good predictors of enhanced aerobic/anaerobic respiration. Fish feces and waste feed are rich in carbon, nitrogen and other elements and are readily available to microbes. Mayer et al. (1995) have developed a method of estimating the trophic availability of enzyme-hydrolyzable amino acids (EHAA) in sediments to typical deposit-feeding macrofauna based on an assay which mimics the natural enzyme pathways. Linton and Taghon (2000) used this measure to show that *Capitella* sp. grew at rates proportional to natural EHAA levels up to ~4 $mg \cdot g^{-1}$ dry sediment. Median values of 22.4 mg hydrolyzable amino acids$\cdot g^{-1}$ dry sediment were found in farm footprints in the Bay of Fundy vs. 1.5 $mg \cdot g^{-1}$ at nearby reference sites (Wildish et al. 2003).

SEDIMENTARY ENRICHMENT

Organic matter preferentially accumulates in net depositional sediments (Newell 1970), and the median particle diameter of soft sediments inversely correlates with organic carbon content. Another way of viewing this is that sediments with a high proportion of fine particles will often have a high carbon content. Small silt/clay particles also have a higher total surface area than an equal weight of large particles (e.g. sand). Thus, there is a positive linear relation between sediment surface area (as $m^2 \cdot g^{-1}$) and the organic carbon content of the sediment, due both to the adsorption of organic matter to sedimentary surfaces, and hydrodynamic sorting of organic matter and mineral particles (Mayer 1989).

Within sediment pore water, the measure used to indicate the balance between oxidizing and reducing agents is the electromotive potential difference between the platinum and reference electrodes (Whitfield 1971). Thus, negative redox potentials indicate a reducing and positive ones an oxidizing sediment. In soft, depositional sediments receiving natural background levels of sedimentation, the sediment-water interface is aerobic and redox potentials follow the general seasonal patterns of temperature in aerobic seawater (Wildish et al. 2002). There are exceptions to this, as where the water column becomes hypoxic or where natural accumulation of organic matter (e.g. wrack transported by onshore winds) occurs. Natural sedimentation rates were reviewed by Hargrave (1985) and found to be generally within the range of 0.1-1.0 g $C \cdot m^{-2} \cdot d^{-1}$ in the World's oceans. The oxic interfacial sediment layer may contain microniches where sulfate reduction occurs (Jørgensen 1977). Deeper in the sediment, the supply of oxygen (by diffusion and aided by macrofaunal burrowing and irrigation) becomes exhausted and conditions become anoxic. This transition is often sharp, as can be seen from lighter coloured surface and deeper, black sulfide containing sediments. The transition area is referred to as the redox potential discontinuity, or RPD, by Fenchel and Reidl (1970), and is where redox potentials rapidly change from positive to negative. Electron sinks other than oxygen are utilized by microorganisms below the RPD, in the following order: nitrogen, sulfur and carbon (Poole and Wildish 1979). Sulfur, in the form of sulfate present in seawater, is utilized by the strictly anaerobic sulfate reducing bacteria (Fenchel and Reidl 1970). The reduced byproduct generally exists in sediments as a metal sulfide. If sulfate becomes locally depleted, carbon compounds are reduced by methanogenic bacteria to produce methane as a byproduct (Martens and Berner 1974). To summarize, depositional sediments receiving natural background levels of sedimentation have an aerobic layer at the interface that contains abundant macrofauna and meiofauna (Fig. 5B). Macrofaunal burrows may extend into the anaerobic layer beneath the RPD.

By contrast, soft, depositional sediments receiving a continuous elevated level of sedimentation (>1g $C \cdot m^{-2} \cdot d^{-1}$, Table 2), principally due to fish feces and waste feed, become anoxic at the interface and develop a black, top-down sulfide layer (Fig. 5A). Sulfide blackening occurs at the surface due to the deposition of metal sulfides stored within the sediments. Some of the sulfide produced may also be oxidized by other chemoautotrophic bacteria (Jørgensen 1977; Howarth 1984; Lumb and Fowler 1989): for example, *Beggiaotoa* sp., which requires both a source of sulfide and oxygen. The white patches of these bacteria may be seen within the surface layer (Fig. 5Ac). This suggests that this particular farm footprint was accumulating feces and waste feed much faster than bacterial metabolism could handle the organic input. There were few macrofauna present in the farm footprint, whereas in a nearby reference location (Fig. 5B), evidence of bioturbating activity of macrofauna was abundant, both above and below the RPD.

A series of mesocosm experiments led Oviatt et al. (1987) to conclude that it was levels of sewage sludge addition >1g $C \cdot m^{-2} \cdot d^{-1}$ that caused hypoxia and marked effects of organic enrichment within the sediment. Hargrave (1994) considered that flux rates of fish feces and waste feed >1g $C \cdot m^{-2} \cdot d^{-1}$ also caused marked organic enrichment effects in net depositional sediments under salmon cages. The process of organic enrichment in farm footprints can reach an equilibrium point on a variable time scale. Depending on site locations, enrichment effects can be reversed if the increased levels of sedimentation are stopped (Brooks et al. 2003). The time scales involved can vary from weeks to years. The latter Pacific coast study suggested that at one site biological remediation was complete within six months. An earlier study in BC (Anderson 1992) showed that at other farm sites biological remediation could take up to 50 months. Other authors, such as Ritz et al. (1989), Johannsen et al. (1994), Karakassis et al. (1999) and Wildish et al. (2001), have suggested various, generally slower, rates of benthic recovery from the effects of intensive fish farming. Present results suggest that site-specific environmental variables will determine the rate of development of organic enrichment, as well as recovery from it. The top-down sulfide layer of depositional sediments at fish farm footprints is characteristic, differing from the classical description of organic enrichment based on sewage and pulp mill wastes (Pearson and Rosenberg 1978, Fig. 6). However, given a stable, high input and enough time, the black layer will develop throughout the profile as in the classical case.

The initial effect of a sudden addition of a high flux of readily decomposable organic carbon to sediments is increased metabolism by aerobic bacteria, leading to hypoxia and anoxia causing death of the most susceptible aerobic life forms (Poole et al. 1978; Pearson and Rosenberg 1978; Gray et al. 2002). In the fish cage footprint, the bulk of the metabolism is by sulfate reduction at a rate much higher than reference stations (Holmer and Kristensen 1992) and results in the loss of nitrification and denitrification pathways (Kaspar et al. 1988). The death of burrowing macrofauna leads to a rapid decline in the capability for irrigation of aerated water within the upper profile and a more rapid development of anoxia. The predominant bacteria become anaerobes, principally sulfate reducers and methanogenic bacteria. The competition between sulfate reducing and methanogenic bacteria for intermediate substrates such as acetate, hydrogen and carbon dioxide is described and the environmental factors affecting the competition adduced by Heyer et al. (1990). Methane is the most stable of the gases produced during anaerobic respiration and H_2S is oxidized in oxic seawater. Methane makes up the bulk of the outgassing from heavily impacted farm sediments (Samuelsen et al. 1988; Wildish et al. 1990). It has been shown recently that a poorly studied group of bacteria, the Archaea, can reduce sulfate and oxidize methane in sediments (Orphan et al. 2001; Thomsen et al. 2001). One of the byproducts of sulfate reduction is the toxic hydrogen sulfide; its production is seasonal, and it can reach high enough levels to kill the sulfate reducers themselves (Poole et al. 1977; Marvin-Pasquale and Capone 1998). It is possible that such an event had occurred in the sediment whose profile image is shown in Fig. 5A. A few opportunistic macrofaunal species are tolerant of low DO and relatively high levels of hydrogen sulfide: for example, the sludge worm, *Capitella capitata* (Tsutsumi 1990) and a few species of bivalves which live in the transition zone between the aerobic and anaerobic zones of the sediment (Savrda and Bottjer 1987). Diaz and Rosenberg (1995) list the species resistant to moderate and severe hypoxia. In addition to *C. capitata*, Brooks and Mahnken (2003) identified the polychaetes *Schistomeringos* sp. and *S. tentaculata* and the crustaceans *Nebalia pugettensis*, *Aoroides* sp. and *Pseudotanais oculatus* as opportunistic species capable of surviving in relatively high sulphide conditions near BC fish farms.

ORGANIC ENRICHMENT INDICES

The two required elements of successful methods to determine the amount or extent of organic enrichment in sediments are scientific rigour and practical resource management relevance (Holmer et al. 2001). Organic enrichment indices may be regarded as proxies of the ecosystem to indicate extent or amount of the effect of organic enrichment. For scientific rigour this means defensibility and providing a means of testing the weight of evidence statistically; and for management relevance, to provide endpoints to trigger management or regulatory actions cost-effectively.

The most widely accepted index is based on macrofaunal species, abundance and biomass (SAB, see the generalized diagram of Pearson and Rosenberg 1978), but their visual representation (reproduced here as Fig. 6) includes other effects of enrichment, such as the position of the RPD and amount and sediment depth of macrofaunal burrowing. However, the position of the RPD is difficult to evaluate in sediments consisting of dark particles (e.g. from basaltic rocks). The Pearson and Rosenberg organic enrichment gradient diagram (Fig. 6) is based on quantitative macrofaunal sampling and can be converted to four significant groupings based on the species and density of macrofauna present along the organic enrichment gradient, useful in managing sediment conditions. A similar concept of stabilization of organic matter along a continuous spatial gradient from a single point source (Poole et al. 1978) also suggested four groupings based on macrofaunal numbers of species and density, dissolved oxygen levels, biodegradable carbon, heterotrophic and autotrophic microbial biomass (Table 4). Brooks and Mahnken (2003) found that the number of infaunal taxa was inversely related to free sediment sulfides in fish farms from the Pacific coast. The DEPOMOD model (Cromey et al. 2002) uses the Infaunal Trophic Index (ITI) developed by Word (1978 in Cromey et al. 2002) to link organic enrichment from fish farms to biological effects. The ITI method has been criticized by Maurer et al. (1999) who believe that "it has achieved an unwarranted level of probity in regulatory reviews."

These indices suffer because the macrofaunal surveys required to determine them are so expensive in time to obtain. Three additional methods have been proposed (Table 4), which can be linked to spatial or temporal versions of the organic enrichment gradients based on macrofauna. They include sediment profile photographs of the top few to 40 cm of the sediment profile, and interfacial sedimentary redox and sulfide measurements in which the four groups can be defined numerically. The methods are: (1) the organism-sediment index (OSI) which includes weighted scores for mean RPD depth, presence/absence of methane and DO in bottom water, and macrofaunal successional stage. (2) the benthic habitat quality index (BHQ) with scores for mean RPD depth, but otherwise differs in considering only the presence/absence of sedimentary surface and subsurface structures. Rhoads and Germano (1986) do not assign numerical limits for each of the four groupings of the OSI shown in Table 4. The benthic habitat quality index of Nilsson and Rosenberg (1997, 2000) relies on weighted inputs to estimate its score, and this has been criticized because it curtails its statistical properties (Karakassis et al. 2002). (3) Whether the empirical limits determined in the Bay of Fundy by Wildish et al. (2001) for the geochemical method (Table 4) are more universally applicable, is still not clear. Hargrave et al. (1997) showed that, in organically enriched interfacial sediments, there was an inverse relationship between redox and sulfide.

Hargrave (1994) proposed a benthic enrichment index (BEI) based on the product of organic carbon (mmol $C \cdot m^{-2}$) and redox (mV, corrected to the normal hydrogen electrode). The concept is to determine the sedimentation rate from the carbon present in interfacial sediments. Plots of BEI against independently observed sedimentation rates were inversely significantly correlated at sedimentation rates up to 1 $g\ C \cdot m^{-2} \cdot d^{-1}$. Possible disadvantages of the method are: i) it does not account for differential biochemical availability of organic carbon; ii) the relationship between BEI and sedimentation rate <1 $g\ C \cdot m^{-2} \cdot d^{-1}$ is not related and hence not predictive; and iii) the BEI has not been related to the organic enrichment gradient and groupings of macrofauna (as in the Pearson and Rosenberg organic enrichment index).

Cranston (1994) described a geochemical method which is a direct measure of net carbon burial rates. The method requires downcore concentrations of sulfate and ammonium to be determined as indicators of the remineralization rates. Since remineralization is dependant on the inputs of organic carbon, the method is a measure of carbon burial rate. As the gradients are rapidly established, the method measures present-day carbon burial rates. Carbon burial rate determinations require large cores and numerous chemical determinations which are expensive in time to take and work up. But the method appears to be robust and has been tested throughout the world (Cranston 1994) from the deep ocean to salmon cage footprints: a range of 0.000001 to 10 $g\ C \cdot m^{-2} \cdot d^{-1}$. Over this range of sedimentation rates, there is a positive linear relationship between sedimentation and carbon burial rates. Like the BEI, carbon burial rates have not been related to macrofaunal responses and, consequently, lack the practical resource management relevance mentioned at the beginning of this subsection.

Recently, Dell'Anno et al. (2002) has suggested a suite of environmental variables to assess the organic enrichment status of the coastal zone in the Mediterranean Sea, based on interfacial sedimentary variables. They include sediment water content and porosity, fluorometric measures of plant pigments, total organic matter and organic carbon (by combustion at 450°C), as well as biochemical measures (including albumin, glucose and tripalmate equivalents), which are converted to carbon equivalents to indicate their availability as biopolymers.

ECOLOGICAL EFFECTS OF ORGANIC ENRICHMENT

The effects of organic enrichment and its ecological consequences have been reviewed (e.g. Poole et al. 1978 and Pearson and Rosenberg 1978, in relation to pulp mill and municipal sewage, and more generally in recent times by Diaz and Rosenberg 1995, Cloern 2001 and Gray et al. 2002). There are also a number of reviews with a focus on marine aquaculture (e.g. Black 2001). Two important generalities emerge from this work:

- the ecological response is complex, involving pelagic benthic coupling and both water column and sediments; and
- the effects are due to all sources of organic matter, both natural and anthropogenic.

Hypoxic/anoxic environments are known (Diaz and Rosenberg 1995) from the geological record and are thus natural phenomena, not solely linked to recent human population explosion and industrialization. Enclosed basins such as the Baltic and Black Seas and a few silled fjords in BC (e.g. Howe Sound (Levings 1980) and Saanich Inlet (Jamieson and Pikitch 1988)) experience severe hypoxia in bottom water, which is sometimes sufficient to cause asphyxiation in benthic macrofauna and fish. Because there is evidence of an increase in the degree and extent of severe hypoxia in some regions in recent times, it is also clear that anthropogenic organic enrichment is a contributing cause (Diaz and Rosenberg 1995).

EFFECTS ON BENTHIC POPULATIONS AND COMMUNITIES

Because of the different physical and biological conditions on the Atlantic and Pacific coasts of Canada, the ecological effects of aquaculture are different. Thus, in Atlantic Canada, the Bay of Fundy salmon culture industry does not usually experience severe hypoxia in seawater because of energetic tidal mixing. Hypoxia, if present, is in near-field sediments and localized to the benthic footprint area. Occasional hypoxic events in the fall are linked to poorly flushed bays and phytoplankton blooms that die there and provide a readily decomposable source for aerobic bacteria (Martin et al. 2001). Microbial changes in the sediment, which cause the appearance of a top-down sulfide layer, result in the death of the resident macrofauna, leaving an azoic sediment or one partially recolonized by pioneering species such as *Capitella capitata* (Wildish et al. 2003).

On the Pacific coast, fish farms are located over deeper and more diverse seabed habitats than in southwest New Brunswick. As mentioned above, most tenures include a mosaic of sediment types (mud, sand, gravel, cobble and mixtures). Rock terraces, cliff walls and boulder fields are also under fish farms in some locations (see Levings et al. 2002 and references therein). It therefore follows that organic enrichment effects from BC farms are relevant to a wider variety of invertebrate species, including epifaunal as well as infaunal forms. In particular, the smothering of suitable larval settlement sites on rocky substrates is an issue which has not been considered in studies to date.

The general response of macrofaunal populations and communities to organic enrichment gradients is well established: it involves the local extinction of the resident equilibrium community, followed by the re-establishment of opportunists if conditions improve, such as capitellids, as primary colonizers. Recolonization and succession of macrofauna affected by organic enrichment from fish farms has been followed by Lu and Wu (1998). Some species are more resistant to hypoxia than others (see Table 2 in Diaz and Rosenberg 1995) and, in general, the crustaceans and echinoderms are most sensitive to the effects of hypoxia. According to the latter authors, species resistant to severe hypoxia include *Arctica, Astarte, Mytilus edulis, Streblospio benedicti* and *Heteromastus filiformis.*

Pearson and Rosenberg (1978) have described macrofaunal changes associated with the organic enrichment gradient which can have a spatial or temporal axis (Fig. 6):

- azoic - with the highest input of carbon and no macrofauna;
- the peak of opportunists - with few, small species in large numbers;
- the ecotone point - where the abundance is low and evenness diversity is high;
- the transitory zone - where large fluctuations in population numbers occur during successional stages, and species diversity increases; and
- the normal or equilibrium population, usually of high diversity, in which sedimentation rates are within the natural range.

Traditional benthic sampling methods, involving grab, sediment sieving, identification of taxa and analysis of community structure (including species diversity, similarity indices and multivariate statistics) have been used to infer disturbance events due to aquaculture (e.g. Rosenthal and Rangeley 1989; Weston 1990; Findlay et al. 1995; Karakassis and Hatziyanni 2000; Pohle et al. 2001). Meiofauna have also been studied in relation to salmon farming in the Bay of Fundy (Duplisea and Hargrave 1996), and it was found that because they are more tolerant of hypoxia than macrofauna, they contribute a greater proportion of benthic community respiration in organically enriched sediments under cages. The thiobiotic meiofauna (Powell 1989) are sulfide insensitive and can aerobically respire at very low DO levels.

CAUSALITY OF DEATH OF MACROFAUNA

Diaz and Rosenberg (1995) have reviewed the observational and experimental data relating low DO levels and H_2S to macrofaunal invertebrate mortality in sediments. Field studies, where care was taken to measure the actual DO experienced by the macrofauna within the benthic boundary layer, suggest that where seasonal hypoxia occurs, levels of 1 $mL \cdot L^{-1}$(~15% of air saturation) begin to cause death. At levels of 0.5 $mL \cdot L^{-1}$ (~7% saturation), mass mortalities occurred. In areas of permanent oxygen deficiency, the benthic communities appear to be adapted to an even lower critical DO level. Behavioural responses to near lethal low DOs have also been identified. Thus, for example, bivalves such as *Mya arenaria*, in a Danish fjord, extended their siphons 20-30 cm into the water column (Jørgensen 1980). This, and similar behaviours documented in Diaz and Rosenberg (1995), are seen as adaptations to hypoxia since the seawater higher in the water column may well be more oxic. Although very low DOs are the major limiting factor for macrofauna, the role of H_2S (and ammonia) in toxicity is less clear. In oxic seawater, both these microbial byproducts are readily oxidized, but where severe hypoxia is present, both may be released. Diaz and Rosenberg (1995) reviewed work that suggested that sediments containing sulfide caused significant additive toxicity. It is of some interest that vertebrate macrofauna, such as fish, have much higher DO requirements for growth, reproduction and survival (Davis 1975) than do invertebrates.

EFFECTS ON ECOSYSTEM FUNCTIONING

Relatively little is known about the consequent effects of organic enrichment to the ecosystem as a functioning process. One simplistic approach is to assume that all of the secondary benthic production which becomes anoxic or hypoxic (and where primary colonization by opportunists is considered to be unavailable to commercially valuable species) is lost to the next trophic level of predators. By calculations based on the area of a typical farm (see p. 23-24): a single cage area = 1.96×10^{-3} km^2, with 14 cages the total area is 27.5×10^{-3} km^2. Given that the secondary benthic production in the area of the Bay of Fundy where the mariculture industry is located is a mean of 21-68 g dry weight $m^{-2} \cdot y^{-1}$ (Wildish et al. 1977), then the total production unavailable to predators is 577-1870 kg on an annually recurring basis for each farm. These calculations ignore the gradient effects described by Pearson and Rosenberg (1978) downstream of the footprint. Individual predators are adaptively programmed only to prey on a specific set of a few species in an equilibrium (=climax) community from among all species lost due to hypoxia. In the Bay of Fundy, the demersal commercial species of interest to resource managers would include groundfishes, scallop, lobster and sea urchins. Very little is known about the effects of organic enrichment on these valued fishery resource species.

From the partial carbon budget shown in Fig. 2, it is clear that azoic or anoxic sediments could cause a significant shift in pelagic-benthic coupling. The effect of organic enrichment in sediments is to move the system to one dominated by bacteria, ciliates and meiofauna, and where the trophic links to the next level of the food web (e.g. to groundfish) are broken. If it is filter-feeding organisms, such as mussels, which are the victims of hypoxia, then the well established feedback loop (Dame et al. 1980) is broken. The feedback loop consists of mussels feeding on phytoplankton and producing plant nutrients as wastes from digestion, which fuels further primary production. It is expected that the loss of a filter-feeder population will destroy the feedback loop and, hence, affect primary production. This is because a bacterially-dominated sediment recycles nutrients within the sediment matrix, releasing nutrients to the water column, such as ammonia, which are less useful as plant nutrients. Although the severely hypoxic areas caused by fish farming in the Bay of Fundy are relatively small, we know of no studies that have examined benthic pelagic coupling in the vicinity of fish farms.

The macrofaunal community analysis presented by Pohle et al. (2001) in bays heavily occupied by salmon farms, but at a distance of ~0.5 km away from them ("reference stations"), demonstrated that significant structural change in macrofaunal communities occurred in an area with a high density of salmon farms. Although the cause could not be identified, the most probable explanation is that an aspect of enrichment linked to salmon farming caused changes in benthic-pelagic coupling in such a way as to exclude some species and encourage others. The cause does not appear to be linked directly to hypoxia as with the cases considered above, and suggests that there is another way that organic enrichment can affect benthic macrofauna.

TOWARDS PREDICTIVE MODELS

Prediction of benthic organic enrichment from fish farms requires the application of mathematical models. There are two major classes of models for addressing such environmental issues: (1) process-oriented models, and (2) empirically-based models. Process-oriented, or mechanistic, models are concerned with constructing mathematical frameworks which describe the major physical and biogeochemical components and the processes through which they interact (i.e. quantifying cause and effect). Empirically-based models use statistical descriptions of the relationships between observable quantities that are indicators for key environmental components or processes. Since our knowledge of how environmental systems operate is limited, process-oriented models will include a variety of empirically motivated parameterizations. Similarly, empirically-based models use accumulated theoretical knowledge to guide the construction of statistical relationships among variables. However, the real test of any model is its predictive skill or its ability to match observations.

PROCESS-ORIENTED MODELS

There is a hierarchy of process-based models that link the production of organic matter in fish farms to benthic organic enrichment. The major physical processes described by these models were outlined in the section on transport and dispersion of organic matter (p. 18) and include water circulation and mixing, as well as suspended sediment dynamics. The biogeochemical processes that act to transform organic matter in the sediment were also briefly mentioned in that section and outlined in more detail in the section on organic enrichment as a process (p. 22). The current state of these mathematical models is briefly discussed below.

Ocean circulation models provide predictions of the temporal and spatial evolution of the three-dimensional currents and water properties variables (i.e. temperature, salinity and density). Such information provides the foundation for predicting the transport and mixing in the coastal zone, including the dispersion of organic matter from fish farms. Examples of ocean models include the Princeton Ocean Model (Blumberg and Mellor 1987) and Canadian version of the CANDIE ocean model (Sheng et al. 1998). These geophysical fluid dynamic models are comprehensive ocean models and have been successfully applied to coastal waters. They provide the basis for linking physical models with marine ecological processes (Hofmann and Lascara 1998). Simplified hydrodynamic models are also frequently used for site-specific coastal engineering problems (e.g. AquaDyn modelling software). These generally concern themselves with relatively simple flows, such as those having no density variations; they have a particular concern with geometry of the system and predicting the effect of fixed structures on flow fields.

Generally, the information from the larger scale ocean models provides the far-field inputs needed for the application of localized, site-specific models.

Sediment transport models predict the time evolution of the spatial distribution of suspended particulate matter, as well as the exchanges of material between the water column and the benthos (e.g. van Rijn 2001). These have their foundation in hydrodynamic models, which provide the velocity and turbulence fields necessary to predict the movement of sediment (Dyer 1986). Such models range from relatively simple one-dimensional models (Jones et al. 1996) to fully comprehensive sediment transport and water quality models (Hamrick 1992). Comprehensive modelling systems incorporate three-dimensional water transport and mixing, mechanistic descriptions of deposition and resuspension, multiple particle size classes of both cohesive and noncohesive particles, and spatially variable bed properties. They have achieved a high degree of sophistication and are increasingly being marketed as software tools for solving applied problems in coastal science and engineering. They include, for example, MIKE-3 of the Danish Hydraulic Instititute and TABS-MD of the US Army Corps of Engineers. For more scientifically oriented studies, there is interest in developing a community model for sediment dynamics (Sherwood et al. 2000).

Organic matter, such as that produced by fish farms, is reworked biologically and chemically in both the water column and the benthos (see Wotton 1994; Heip et al. 1995). In the water column, particulate organic matter plays an important role in the aquatic food chain. It is remineralized by bacteria to inorganic nutrients, wherein it can enter the planktonic food web and may directly provide a food source for zooplankton. Organic detrital matter is therefore included as a key component in the widely used lower trophic level ecosystem models of pelagic biogeochemistry (Fasham 1993). With respect to benthic organic enrichment from fish farms, we are frequently dealing with shallow seas where benthic-pelagic coupling is important. For instance, in many nearshore areas, a substantial fraction of organic matter may settle directly to the sea bottom, effectively untransformed from its generating site. This organic input to the benthos drives a variety of biogeochemical processes (see p. 23). Soetaert et al. (2000) review a hierarchy of models for describing these processes. The most realistic, as well as the most complex, of these models are the diagenetic models which couple water column processes to vertically resolved sediment biogeochemical models (Boudreau 1997; Wijsman et al. 2000). A variety of simplified versions of these models is also used to represent benthic-pelagic coupling in ocean biogeochemical models.

EMPIRICAL MODELS

The material above provides a brief list of the types of models needed for a comprehensive assessment of the physical and biogeochemical processes contributing to benthic organic enrichment. Due to the complexity inherent in any application of this hierarchy of models, a variety of much more simple models has been proposed in order to more directly address the issue of the benthic effects of fish farm waste. As an example of an empirically-based model, Findlay and Watling (1997) proposed an oxygen-based framework for assessing the benthic response to organic enrichment from salmon net pen culture. This model was based on the balance between benthic oxygen supply (via bottom currents and sediment diffusion) to oxygen demand (as driven by the rate of organic input). Morrisey et al. (2000) applied this model to marine fish farm sites in New Zealand and found good agreement with observations. An example of a more process-oriented approach is given by Dudley et al. (2000) where dispersion of wastes from a fish farm to the benthos is estimated using a transport model. A hybrid approach to the modelling of benthic organic enrichment effects is used by DEPOMOD (Cromey et al. 2002). It uses a particle tracking model in a simple flow field to predict the spatial distribution of the amount of solids accumulated on the sea bed. It then links these changes to benthic community structure using empirical relationships. In BC, Carswell and Chandler (2001) and Stucchi (2004) have developed empirical particle tracking models that give estimates of size of the sediment field under and near fish farms, but these models are not yet linked with benthic biological data.

From our experience with the Bay of Fundy salmon culture industry (Rosenthal and Rangeley 1989; Wildish, personal observation), we know at least three farms which have been abandoned due to negative environmental conditions. This included low dissolved oxygen levels that reduced the growth rate of salmon. All three sites shared very poor flushing characteristics, net depositional sediments, shallow depths and a very high input of readily available carbon in the form of feces and waste feed to the farm footprint. The situation is visualized in Fig. 7, where U is the mean current velocity in $cm{\cdot}s^{-1}$, Z the depth in m and C the sedimentation rate in g $C{\cdot}m^{-2}{\cdot}d^{-1}$. All three farms mentioned above would cluster within the dark shaded inner cone, where footprint sediments are anoxic at the sediment-water interface. This would be equivalent to organic enrichment stage 0 (anoxic, see Table 4). Approximately 15% of 53 farms monitored in the Bay of Fundy in 1992-93 classify in, or close to, the most enriched category (Table 4) during the annual fall environmental monitoring program (Thonney and Garnier 1993; Anon. 2001). Thus these farms would be clustered within the inner cone of Fig. 7.

TOWARDS METHODS OF MONITORING ORGANIC ENRICHMENT

Because of the intimate connections between the water column and sediment inferred from benthic-pelagic coupling, it is important to include both seawater and sediment in a rational monitoring program to detect organic enrichment. Thus, in this section we consider both near- and far-field phenomena. We hope that Tables 5 to 8 will allow the reader to make his/her own rational choices of methods which take account of the purpose of monitoring, the geographic area to be covered, practical limitations on sampling of different substrates, as well as the cost-effectiveness of each method.

TYPES OF MONITORING

The general types of monitoring to detect organic enrichment in the marine environment can be recognized (Table 5) by their purpose. The site comparison method tests the null hypothesis that selected reference and treatment sites are not drawn from the same sample area. Reference locations are selected to have similar environmental characteristics as the treatment ones (e.g. sediment type, hydrodynamics, macrofaunal community, etc.) and are close to the treatment site, but outside the zone of enhanced sedimentation. The purpose of the temporal method is to test the null hypothesis that a treated site does not become different from a reference site during the passage of time. The purpose of a geographical effects study is to determine the spatial limits of organic enrichment and to test the null hypothesis that the reference condition is present throughout the study area. In those cases where multiple organic enrichment sources are known to be present, it may be required to identify the source by chemical or biochemical means. Lastly, the purpose of practical monitoring is to determine the magnitude of organic enrichment in order to initiate remediation measures. Practical monitoring is not associated with a hypothesis; although by the addition of reference sites, it could be transformed to the site comparison method.

In working with each monitoring method, it is necessary to know the fraction of the ecosystem to be sampled in relation to the size of the sampling device. Because of the fundamentally different ways that seawater and sediment are sampled, the monitored amount is volume for the former and area for the latter. Provided in Table 6 is an example for sediments of typical relationships between the sampled area and appropriately sized samplers. A comprehensive way to compare one aspect of the sampling limitations of each method for sediments is as area sampled per unit time (Kenny et al. 2000), as is shown in Tables 7 and 8.

A frequently encountered problem in benthic monitoring is that the type of substrate to be sampled cannot be accessed by the sampler (e.g. a grab cannot be quantitatively used on a hard, rock surface and also cannot sample mixed substrate such as sand/gravel or shell hash effectively). Thus it may be necessary to use an otherwise less satisfactory sampling method on coarser substrates, such as underwater (U/W) photography or video photography. These methods, however, have disadvantages because it can be difficult to detect microhabitat effects in the nooks and crannies of boulder fields (Bright 2001), and obviously no seabed sample is obtained for chemical and biological analysis.

Two important characteristics of monitoring methods are to know at what stage of development the method has progressed, as well as knowledge of how well it is accepted by the scientific community. For the latter, the following designations are adopted for Tables 7 and 8:

*	under development, not accepted;
**	developed, but not accepted;
***	developed and fully accepted.

SEAWATER

Organic enrichment in the euphotic zone of the marine environment is referred to as eutrophication. The concept of trophic status in marine surface waters is borrowed from the successes achieved by freshwater biologists (see Vollenweider and Dillon 1974). As a process, it involves hypernutrification, increased microalgal biomass, increased turbidity and changes in the DO balance. It is not surprising that the methods that have been tried and tested to characterize eutrophication are based on pelagic-benthic processes described above and include: phytoplankton species x abundance matrices, chlorophyll a as an indication of microalgal biomass, turbidity, plant nutrient and DO concentrations (Table 7).

Because no single variable shown in Table 7 can be accepted as the single, true indicator of the trophic status of surface seawater as oligotrophic, mesotrophic or eutrophic, multiparameter classifications have been sought which use all or most of the data from the listed methods. The preliminary OECD classification of Vollenweider and Kerekes (1982) was based on chlorophyll a, plant nutrients (total P and N) and Secchi depths, and showed considerable overlaps for each trophic group if standard deviations of the OECD data were considered. A recent attempt (Zurlini 1996) to provide trophic classifications using a probabilistic approach has shown that the classification uncertainties are basically caused by the, often unknown, hydrodynamics in the area of concern. Strain and Yeats (1999) used chemical methods (plant nutrients, DO and trace metals) in 34 marine tidal inlets of the Canadian east coast to provide a ranking of them along the eutrophic gradient, but which was not related to classical trophic groups or their biological characteristics. Satellite remote sensing can map areas for plant pigment distribution patterns in shallow sea surface waters at very fast rates (Kenny et al. 2000).

SEDIMENT

Among the best known methods shown in Table 8 are those based on classical macrofaunal sampling and analysis, although these are also the most costly because of the time required to identify samples to the lowest possible taxon. Alternative methods such as sediment profile imaging or geochemistry are much more cost efficient at defining the continuum of the organic enrichment index. The presently used traditional macrofaunal sampling methods by grab and corer cannot be used to map areas larger than a few km^2. This is because of the limited sampling area of grab or corer, the patchiness and complexity of soft sediment habitats and the long work-up time required for each sample. Among the recently developed methods included in Table 8 are aerial photography of the littoral, U/W video photography and multi-beam acoustics of sublittoral sediments. All of these have the potential to produce detailed and accurate maps over much larger geographic areas than by grab sampling. However, all require much more research to reach their potential as mapping tools. The main requirement for this work of the future is extensive groundtruthing with available traditional methods. As an example, acoustics may be useful in mapping of benthic communities, trophic groups or secondary benthic production (e.g. Wildish et al. 1998; Kostylev et al. 2001).

SELECTION OF MONITORING METHODS

Consideration should be given to the projected amount of organic waste in relation to the volume of seawater and surface area of substrate. Other factors considered would include the sediment characteristics and hydrodynamic environment to determine how broadly the wastes will be dispersed. Local knowledge of the presence/absence of toxic microalgae might also enter these considerations.

For benthic monitoring, the presence of predominantly hard or soft substrates will dictate the type of sampling that can be undertaken. Such considerations can be visualized as a decision tree (Fig. 8). Subsequent choices will depend on the size of the ecosystem that is to be sampled and determined by the primary goal of the study as shown in Table 5. Synoptic survey methods such as remote sensing of chlorophyll a in surface waters, U/W photography of hard substrates, video photography and acoustic surveys for soft sediments, are best in geographical studies (goal 3, Table 5). For the other goals (1, 2 and 5, Table 5), there are available a few alternative methods, which can be chosen on the basis of cost-effectiveness (Wildish et al. 2001). This assumes that each of the candidate methods is scientifically defensible and capable of providing a test of statistical validity for the hypothesis tested. Thus, geochemical monitoring could replace the classical species x abundance approach in Table 8 because it is more cost-effective at least in goals 1 and 5 (Wildish et al. 2001). Grizzle and Penniman (1991), in a local geographic study (goal 5, Table 5) along an organic enrichment gradient, found that sediment profile images (SPI) were as useful as the classical macrofaunal method in delimiting the spatial extent of enrichment. On the other hand, Rumohr and Karakassis (1999) found that SPI was not a surrogate for classical benthic monitoring with macrofauna in a temporal study (goal 2, Table 5), although their data lacked a pronounced organic enrichment gradient.

Where practical monitoring (goal 5, Table 5) is required, there is need to produce data with clear thresholds that can trigger remediation actions. For seawater, universally applicable phytoplankton species indicators of organic enrichment are not available. It is similarly difficult to use chlorophyll a concentrations to indicate eutrophic conditions, because of the variability between different areas. For DO, the physiological response for commercially valuable species (e.g. cultured finfish) can be used to set specific DO concentrations which trigger remediation action. For soft sediments, macrofaunal indicator species and multivariate analysis of species x abundance matrices have been highly developed and are suitable for most of the goals shown in Table 5 (Pearson and Rosenberg 1978; Clarke and Green 1988; Warwick 1986, 1993). The four stages of the organic enrichment gradient can be determined by at least three independent methods: macrofaunal, SPI and geochemical. It is not yet clear whether the concordances among these methods apply universally (see Wildish et al. 2001). Nevertheless, they do provide the local criteria to trigger remediation or environmental corrective action before the sediments become azoic.

Cogent criticisms of macrofaunal sampling and analysis are presented by Rhoads and Germano (1986) and include the following:

- such methods are expensive in time (to name and sort the macrofauna), and grab sampling and sieving destroys most of the ecological evidence of organic enrichment; and

- the emphasis on statistical comparisons between treated and reference stations or multivariate analysis to separate populations on the basis of macrofaunal sructure simply determines differences without providing any clues as to their causality.

As pointed out previously, SPI and sediment geochemical methods provide a more cost-effective and credible alternative to the use of macrofaunal sampling and analyses for routine monitoring purposes.

Monitoring methods currently in use in the New Brunswick salmon culture industry (Anon. 2001) were developed by joint provincial/federal consultations with the industry (Janowicz and Ross 2001) and include measures for redox potential and total sulfides. One format for collecting and evaluating environmental data for resource management purposes of fish farms is the decision-support system (Silvert 1994). Using data from Atlantic Canada, Hargrave (2002) presented a worked example of a decision-support approach to aid in determining if aquaculture siting proposals should be supported or rejected. The kinds of input variables or monitoring results needed in such decision-support estimates must be regularly reviewed. This is true generally of monitoring methods, which are continually being improved or updated with better ones. The use of decision-support systems in environmental management methods is considered by Jones et al. (2002).

Operational monitoring regulations and protocols for soft sediment habitats were developed in British Columbia (Anon. 2002a, b) with the following measures relevant to organic enrichment: free sulfides, redox potential, total volatile solids or total organic carbon, and sediment grain size. Biological sampling can be required if sulphide levels exceed prescribed levels. Video monitoring is required for hard bottoms. In support of the scientific basis for operational monitoring, several studies have been carried out by DFO researchers in Pacific Region to investigate the potential environmental effects of organic enrichment on fish habitat productive capacity. Levings et al (2002) listed 12 scientific criteria that can potentially be used to identify sites influenced by waste material. Various combinations of these criteria have been considered in previous studies within the Broughton Archipelago. Levings et al. (2002; Appendix I) investigated an abandoned and poorly flushed site, Carrie Bay, and demonstrated that the benthic fish habitat at this location was affected by the fish farm operations, using the following criteria: area affected, percent silt/clay, sediment carbon/nitrogen, invertebrate fish food abundance, bacterial mats, algal biofilms, likelihood of short term recovery, and zinc levels. Brooks (2004) recently reported that neither chemical nor biological remediation could be considered complete at Carrie Bay after five years of fallowing, supporting the view that fish habitat productivity was impaired by organic enrichment for a substantial period of time.

RESEARCH NEEDS

There are two general types of research needed to fill the knowledge gaps identified by this overview:

- to provide understanding of processes as input to models and environmental monitoring programs (including improved methods); and
- to research those methods needed to assess organic enrichment or near-field effects (e.g. predicting holding capacity).

We have listed our suggestions for the research needed for the above purposes keyed to the section headings used in the review.

PHYSICAL DISPERSION AND SEDIMENTATION

1. The study of dispersion of organic matter from fish farms involves sedimentology and physical and chemical oceanography. These fields provide the foundational knowledge for addressing the scientific measurement of organic enrichment in sediments which arises from finfish aquaculture. There are well established frameworks in each of the above disciplines for addressing organic enrichment from fish farms. Further studies might include:
    - coastal circulation, mixing, dispersion and transport processes in the vicinity of fish farms in support of process models; and
    - observational and modelling studies of water column particle dynamics, such as flocculation and particle-particle interactions.

ORGANIC ENRICHMENT AS A PROCESS

2. Seasonal studies of organic enrichment. A fundamental understanding of the temperate marine environment (where salmon grow-out is carried out) is a prerequisite for the rational management of fish farming. This is for two reasons: first, to understand how ecological factors, not in the direct control of the farmer, affect the salmon growth and production cycle (see Peterson et al. 2001 for a start in this direction); and second, how ecological factors affect organic enrichment events resulting from salmon farming. Specific projects related to the second of these might include:
    - a seasonal study of a heavily organically-enriched fish farm footprint. The aim would be to determine the ecological factors that come into play during the season and particularly under what conditions the self-poisoning by H_2S mentioned earlier occurs. Of interest for monitoring purposes would be the behaviour of redox potential and total sulfide levels during self-poisoning.
    - seasonal studies of redox potential and sulfide over a wide range of sedimentation rates from natural background to heavily organically-enriched to determine if the inverse relationship between redox potential and total sulfide described by Hargrave et al. (1997) persists at all seasons.
    - seasonal studies to show how macrofaunal bioturbation increases the rate of degradation of organic matter. This should include an experimental study of the mechanisms by which macrofauna influence the degradation of fish farm wastes.

3. Measure the availability of carbon to microbial decomposers. A number of possible methods besides EHAA (which is probably too complex a method for routine use) could be used for the purpose of measuring available carbon to the decomposer community (Table 9). The requirement is for a cost-effective method that distinguishes between bio- and geo-polymer carbon and can be used routinely and reproducibly in a wide range of sediment types.

ECOLOGICAL EFFECTS

4. In the Pacific region, determine effects of organic enrichment on infauna living in coarse substrates (sand, gravel, cobbles and mixtures) and epifauna living on boulders and rock walls. These studies should include food chain effects for fish, as well as direct effects on fish spawning habitats in rocky areas (Levings et al. 2002).

5. Determine organic enrichment effects on ecosystem functioning as they affect valued links in the food web. The near-field effects to be expected in the Bay of Fundy would involve benthic fisheries of commercial value (e.g. groundfish, lobster, scallop and sea urchins). There is some evidence (Wildish, unpublished data) that feeding grounds of haddock are damaged if they are in close proximity to salmon farms. The introduction of a salmon farm on Grand Manan near a lobster spawning area resulted in the spawning area being displaced (Lawton et al. 2001). Neither of these preliminary studies can offer experimental proof to establish the cause-effect nature of the relationship. Further detailed experimental and observational studies are required to determine the cause in these and similar cases.

6. Understand how organic enrichment from aquaculture affects benthic-pelagic coupling. Some important questions are:
   - how much of the sedimenting carbon and dissolved organic carbon are transported away from the footprint site?
   - what is the fate of the carbon that remains at the footprint?
   - are there ways (other than extreme hypoxia/hydrogen sulfide toxicity) that the organic enrichment process can alter benthic communities and their functioning?

   The answers to the first two questions will aid in predictive modelling, and the last in explaining the results of Pohle et al. (2001). Possibly mesocosm experiments as described by Oviatt et al. (1987) will aid in answering the last of these questions.

7. Undertake further fallowing studies in Pacific and Atlantic Canada. Further temporal studies to determine the successional stages of recovery of the macrofauna and the rate of recovery of both structure and function of the macrofauna for a representative range of the farm site habitats are needed to confirm the rapid rate of recovery suggested in Brooks et al. (2003).

MODELS OF ORGANIC ENRICHMENT

8. Model verification studies. There is available a hierarchy of models from the most complex process-oriented to simple empirical ones with only a few variables. Collaborative efforts are needed between modeler and field biologist to calibrate, validate and synthesize the information gathered. Specific projects suggested are:
   - undertake research into Lagrangian-based particle models for coastal flow fields. This approach can help establish the link between organic matter at a fish farm and its accumulation in sediments;
   - apply state-of-the-art sediment transport models to the dispersion of wastes from a fish farm;
   - apply state-of-the-art, process-oriented, pelagic-benthic biogeochemical models to finfish farms receiving organic wastes; and
   - investigate the relationship between organic enrichment loading and changes in infaunal and epifaunal communities.

9. Predict holding capacity limits. We believe that it is necessary to distinguish between "carrying capacity" and "holding or assimilative capacity." For the latter, the fish are supported at the site by imported feed, so the feed supply is never limiting (as with the former) and here the limiting factor is considered to be the amount of local or footprint organic enrichment the farm creates. What is required is an inexpensive (therefore a simple empirical) model which can predict the biomass or numbers of fish that can be supported at a potential new site, without causing serious organic enrichment in the footprint. Judging by the lack of effort to validate existing models to predict holding capacity, no fully satisfactory one exists. It should also be realized that such models are limited to the footprint and do not address the far-field effect due to accumulation from many fish farms.

MONITORING ORGANIC ENRICHMENT

10. Develop geographical survey methods. Current benthic methods by grab and corer are too slow to be able to monitor spatially to the extent that will be required for the integrated coastal zone resource management of the future. We believe that a concerted effort should be made to further develop satellite, aerial surveillance, U/W video photography and acoustic methods. The latter is probably the least developed for the purpose of mapping features of biological significance (e.g. benthic macrofaunal production or by the dominant trophic groups), but is considered to have the greatest potential for coastal zone management purposes. Acoustic methods development research, including for environmental monitoring purposes, is part of the SEAMAP proposal.

11. Devise new environmental monitoring methods. Some examples of possible new methods are shown in Table 9. We recommend that the search for improved monitoring tools should be continuing, with the probable improvements occurring in the applicability of the method for difficult-to-sample habitats (e.g. hard substrates) or in cost-effectiveness.

12. Calibrate, standardize and audit (= quality control, or QC) existing monitoring methods. The development of standards, calibration methods and quality control of the individuals and groups using organic enrichment monitoring methods should be given more support than presently.

13. Develop Canada-wide standards (across federal/provincial departments) for the above methods. Part of this should involve developing standardized statistical methods to be used with the standard organic enrichment monitoring methods.

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Table 1. Marine cultured salmon production (weight in tonnes), including steelhead, in Canada. The gross value (Canadian $000) to producers after 1986 is also shown. Data source: DFO Statistical Services.

	Atlantic Canada		British Columbia	
Year	Weight (t)	Value ($'000)	Weight (t)	Value ($'000)
1970			100	
1971			100	
1972			100	
1973			100	
1974			49	
1975			49	
1976			47	
1977			60	
1978			58	
1979	6		48	
1980	13		130	
1981	24		100	
1982	44		130	
1983	72		60	
1984	260		300	
1985	409		320	
1986	774	8,490	400	2,728
1987	1,628	18,689	1,932	12,783
1988	2,981	32,569	6,553	38,859
1989	4,160	43,163	11,883	59,739
1990	7,965	74,232	13,512	81,003
1991	10,252	86,605	24,362	110,913
1992	10,915	89,747	19,814	115,518
1993	11,505	98,593	25,555	138,143
1994	12,522	97,346	23,657	153,815
1995	16,122	120,525	27,275	170,365
1996	18,897	135,587	27,756	155,931
1997	21,256	152,244	36,540	175,944
1998	18,772	133,157	42,200	228,900
1999	29,192	188,238	49,700	292,200
2000	38,718	230,244	49,000	278,400
2001	42,311	219,100	67,700	269,400

Table 2. Organic carbon sedimentation rates under/near salmonid mariculture cages, in units of g $C \cdot m^{-2} \cdot d^{-1}$. Asterisks indicate that the measure assumes that the organic matter or volatile solids measured contains 50% total organic carbon. Based on Hargrave (1994).

Location	Rate	Reference
Norwegian fjord	4-181*	Ervik et al. (1985)
Gullmar Fjord	38-78	Hall et al. (1990)
Scottish sea lochs	23-35*	Gowen et al. (1991)
Japanese coast	12-17*	Kadowaki et al. (1980)
Bay of Fundy farms	?-15	Hargrave (1994)
Norwegian coast	1-15	Hansen et al. (1991)
Maine coastal inlet	5	Findlay et al. (1995)
Nubeena, Tasmania	2-6	Ye et al. (1991)
Scottish Farm A	3.8-5.9*	Cromey et al. (2002)
Scottish Farm B	8.2-16.4*	Cromey et al. (2002)
Broughton Archipelago, BC	5	Sutherland et al. (2001)
BC Farm A	7.9*	Brooks (2001)

Table 3. Mass balance waste estimate of the carbon content of feces and non-eaten feed for a 50-m diameter salmon cage producing mean 5-kg live weight salmon. Calculations assume that all of the sedimentation occurs directly under the farm. Based on data in Peterson et al. (2001) and Ackefors and Enell (1994).

Number of salmon produced	Total wastes, tonnes $C\ y^{-1}$	Sedimentation, g $C \cdot m^{-2} \cdot d^{-1}$
1000	0.80	0.83
2500	1.99	1.66
5000	3.97	3.32
7000	5.6	4.64
14000	11.1	9.28

Table 4. The organic enrichment gradient (after various authors). The organic carbon sedimentation rate increases from left to right.

Type of measure	Successional stages (Nilsson and Rosenberg 1997)				Reference
	III	**II**	**I**	**0**	
Microbial	Normal	Oxic	Hypoxic	Anoxic	Poole et al. (1978)
Macrofaunal	Normal	Transitory	Polluted	Grossly polluted	Pearson and Rosenberg (1978)
Sediment profile imaging					
Organism-sediment index (OSI)	III	II	I	Azoic	Rhoads and Germano (1986)
Benthic habitat quality index (BHQ)	>10	5-10	2-4	<2	Nilsson and Rosenberg (1997)
Geochemical	Normal	Oxic	Hypoxic	Anoxic	Wildish et al. (2001)
Eh, mV_{NHE}	>+100	0-100	-100-0	< -100	
$S^{=}$, µM	<300	300-1300	1300-6000	>6000	

Table 5. Monitoring goals to detect organic enrichment in sediments (based on Wildish et al. 2001). H_o is the null and H_1 the alternative hypothesis.

Goals	Enrichment effect measured	Associated hypothesis
1. Site Comparison	Difference between treatment/reference sites	H_o reference = treatment site H_1 reference ≠ treatment site
2. Temporal	Before/after status	H_o reference = treatment site at t_o H_1 reference ≠ treatment site at t_1
3. Geographical	Limits of impact	H_o reference condition throughout the study area H_1 reference and enriched condition delimited in the study area
4. Source Identification	Source of enrichment	H_o reference = treatment source H_1 reference ≠ treatment source
5. Practical	Relative impact	None, it triggers remediation

Table 6. Categorization of the sampled area in benthic monitoring.

Category	Area, m^2	Examples of samplers used
Ultra microscale	<0.1	Cut-off plastic syringe
Microscale	0.1-10	Grab samplers, core samplers
Mesoscale	10-100	Grabs, video photography
Macroscale	100-1000	Acoustic mapping, video photography
Megascale	>1000	Acoustic mapping

Table 7. Monitoring methods to assess organic impacts in seawater and their suitability (+) or unsuitability (-) for monitoring purposes. The number of asterisks (1-3) indicates the degree of development and acceptance of each method. See text (p. 35) for further information.

Method	Volume m^3	Citation number	Goal 1	Goal 2	Goal 3	Goal 5
Phytoplankton species x abundance matrices	0.1-1000	1, 2, 3 4, 5, 6	+ ***	+ **	- **	- **
Dissolved oxygen concentration	0.1-1000	6, 7, 8, 9, 10, 11, 12	+ ***	+ **	- **	+ **
Plant nutrient concentration – Total nitrogen and phosphorus	0.1-1000	6, 12,13, 14, 15, 16	+ ***	+ **	- **	- **
Turbidity – Secchi disk depth, m	0.1-1000	6, 16	+ ***	+ **	- **	+ **
Microalgal chlorophyll concentration	0.1-1000	1, 6, 12, 16, 17, 18, 19, 20, 21, 22	+ *	+ *	- *	+ *
Aerial remote sensing	>1000	23, 24	+ *	+ *	+ **	+ *

[1] Ankar et al. (1977); [2] Huisman et al. (1999); [3] Sanders et al. (1987); [4] Spencer (1985); [5] Villegas and de Giner (1973); [6] Vollenweider and Kerekes (1982) [7] Cai et al. (1999); [8] Erez et al. (1990); [9] Findlay and Watling (1997); [10] Lee et al. (1991); [11] Trummer et al. (2000); [12] Wildish et al. (1993); [13] Cloern (2001); [14] Strain and Yeats (1999); [15] Vollenweider and Dillon (1974); [16] Zurlini (1996); [17] Aiken (1981); [18] Bianchi et al. (1993); [19] Bianchi et al. (1995); [20] Lucas et al. (2000); [21] Schluter et al. (2000); [22] Spencer (1985); [23] Budd et al. (2001); [24] Leming and Stuntz (1984)

Table 8. Monitoring methods to assess organic impacts in sediments and their suitability (+) or unsuitability (-) for monitoring purposes. Area sampled per unit time, from Kenny et al. (2000). Asterisks as in Table 7.

Method	**Area per unit time, $km^2 \cdot h^{-1}$**	**Citation number**	**Goal 1**	**Goal 2**	**Goal 3**	**Goal 5**
Macrofaunal species x abundance matrices	0.003	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20	+ ***	+ ***	- **	- **
Sediment profile imaging (SPI)	0.001	8, 12, 15, 21, 22, 23, 24, 25	+ ***	+ **	- **	+ **
Sediment geochemistry, redox + sulfide	0.003	9, 20, 26	+ ***	+ **	- **	+ **
Aerial photography of the littoral	10	27, 28	+ *	+ *	+ *	
Benthic video photography of the sublittoral	0.2	29	+ *	+ *	? *	+ *
Acoustic mapping of sediments of the sublittoral	0.8-10	30, 31, 32, 33, 34	+ *	+ *	+ *	+ *

[1]Beukema (1988); [2]Clarke and Green (1988); [3]Craeymarsh (1991); [4]Diaz and Rosenberg (1995); [5]Ferraro et al. (1989); [6]Ferraro and Cole (1995); [7]Findlay et al. (1995); [8]Grizzle and Penniman (1991); [9]Hargrave et al. (1997); [10]Karakassis et al. (1999); [11]Karakassis and Hatziyanni (2000); [12]Nilsson and Rosenberg (2000); [13]Pearson and Rosenberg (1978); [14]Poole et al. (1978); [15]Rumohr and Karakassis (1999); [16]Underwood (1992); [17]Warwick (1986); [18]Warwick (1993); [19]Weston (1990); [20]Wildish et al. (2001); [21]Aiken (1981); [22]Nilsson and Rosenberg (1997) [23]Rhoads and Germano (1982); [24]Rhoads and Germano (1986); [25]Valente et al. (1992); [26]Wildish et al. (1999); [27]Kenny et al. (2000); [28]Klemas et al. (1980); [29]Crawford et al. (2001); [30]Hughes-Clarke (1994); [31]Hughes-Clarke et al. (1996); [32]Tlusty et al. (2000); [33]Wildish et al. (1998); [34]MacDougall and Black (1999).

Table 9. Possible new methods in seawater (W) or sediment (S) applicable to the goals of Table 5. Asterisks as in Table 7.

Method	**Citation number**	**W or S**	**Potential applicability to goals**				
			1	**2**	**3**	**4**	**5**
Microbial biomass by DNA and ATP measure	1	S	*	*	*		*
"Apparent bacterial concentration"	2, 3	W	*	*	*		*
Sterols as indicators of mammalian wastes	4	S				*	
Bioavailable amino acids in sediments	5	S				*	
Sulfide profiles	6	S	*	*	*		

[1]Stoeck et al. (2000); [2]Rodriguez et al. (1992); [3]Schluter et al. (2000); [4]Venkatesan and Santiago (1989); [5]Mayer et al. (1995); [6]Muller and Stierli (1999).

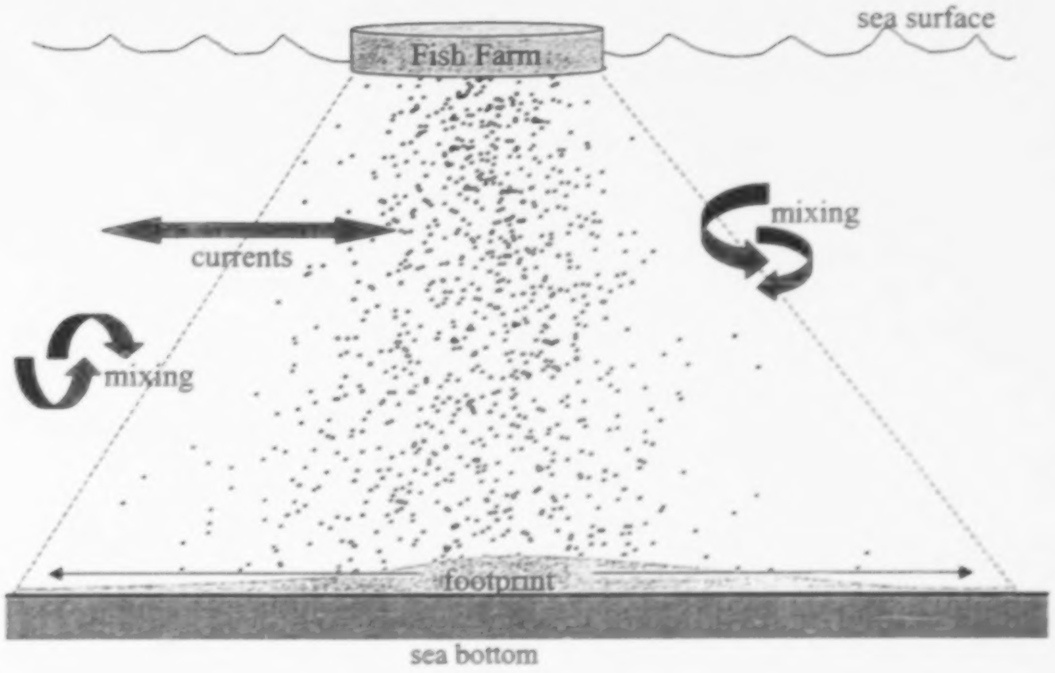

sea surface
Fish Farm
currents
mixing
mixing
footprint
sea bottom

Fig. 1. Conceptual view of the formation of the fish farm footprint from sedimenting fish feces and waste feed.

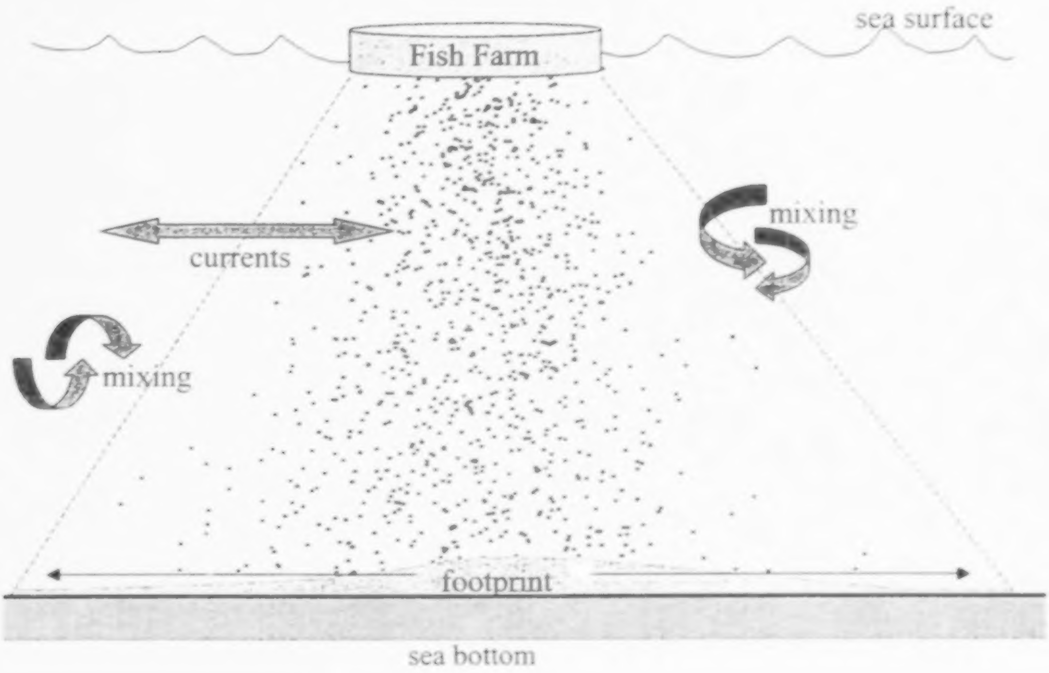

sea surface
Fish Farm
currents
mixing
mixing
footprint
sea bottom

Fig. 1. Conceptual view of the formation of the fish farm footprint from sedimenting fish feces and waste feed.

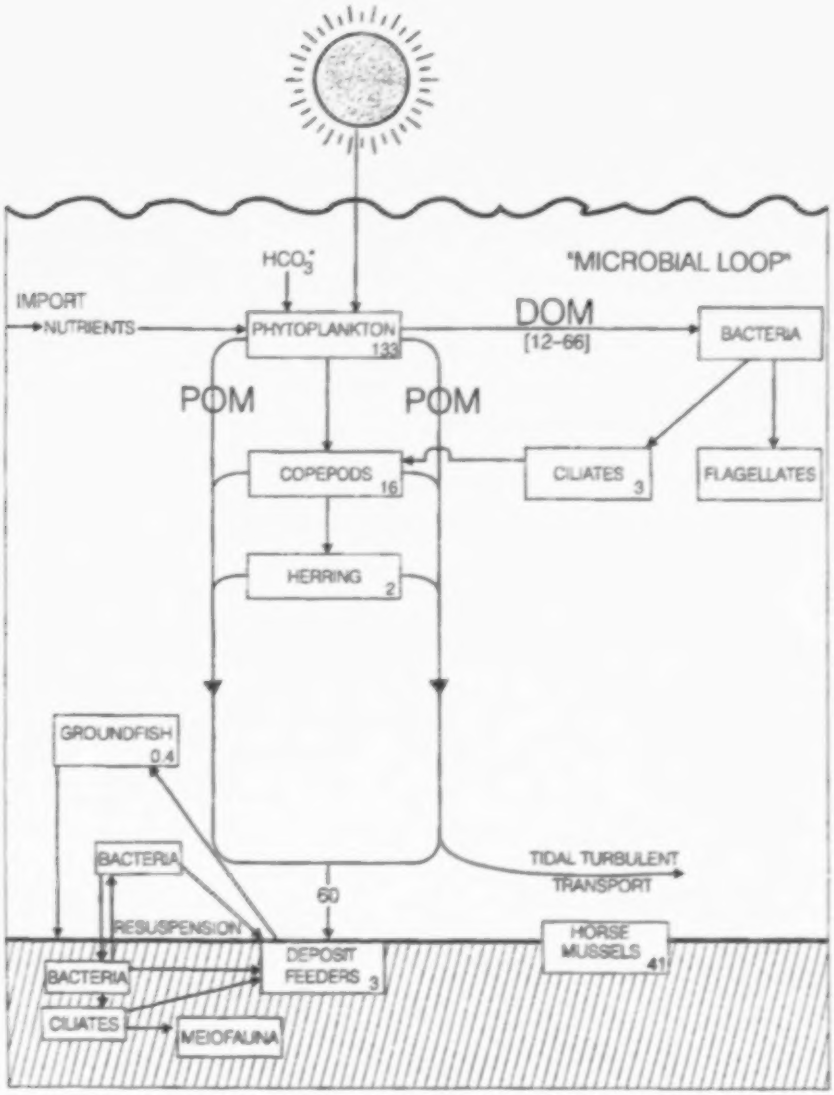

HCO$_3^-$
"MICROBIAL LOOP"
IMPORT
NUTRIENTS
PHYTOPLANKTON 133
DOM
[12–66]
BACTERIA
POM
POM
COPEPODS 16
CILIATES 3
FLAGELLATES
HERRING 2
GROUNDFISH 0.4
BACTERIA
TIDAL TURBULENT TRANSPORT
60
RESUSPENSION
DEPOSIT FEEDERS 3
HORSE MUSSELS 41
BACTERIA
CILIATES
MEIOFAUNA

Fig. 2. Partial carbon budget for an equilibrium community in the lower Bay of Fundy. From Wildish and Kristmanson (1997).

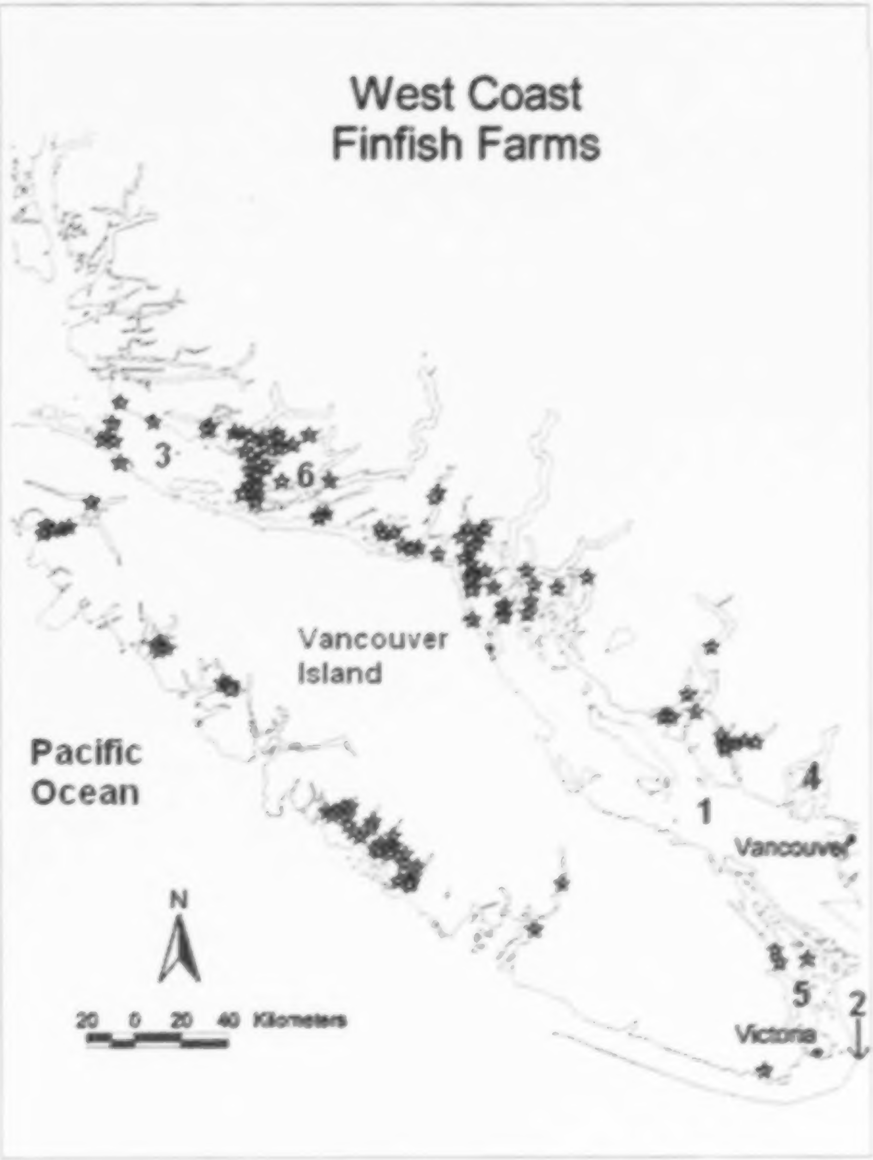
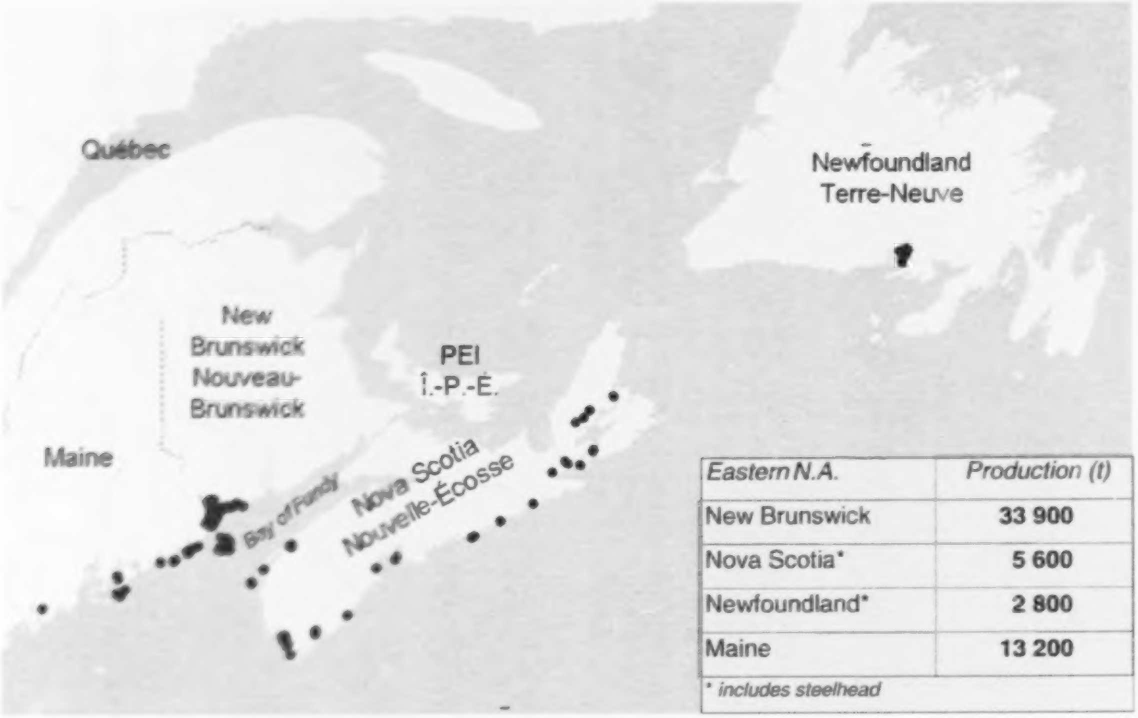

West Coast
Finfish Farms
Vancouver Island
Pacific Ocean
1
2
3
4
5
6
Vancouver
Victoria
N
20 0 20 40 Kilometers

Fig. 3. Chart showing the distribution of salmon farms in British Columbia in 2001.


Québec
Newfoundland
Terre-Neuve
New
Brunswick
Nouveau-
Brunswick
PEI
Î.-P.-É.
Maine
Bay of Fundy
Nova Scotia
Nouvelle-Écosse

Eastern N.A.	Production (t)
New Brunswick	**33 900**
Nova Scotia*	**5 600**
Newfoundland*	**2 800**
Maine	**13 200**

* includes steelhead

Fig. 4. Chart showing the distribution of salmon farms in Atlantic Canada and the United States, with production statistics by province/state in 2001.

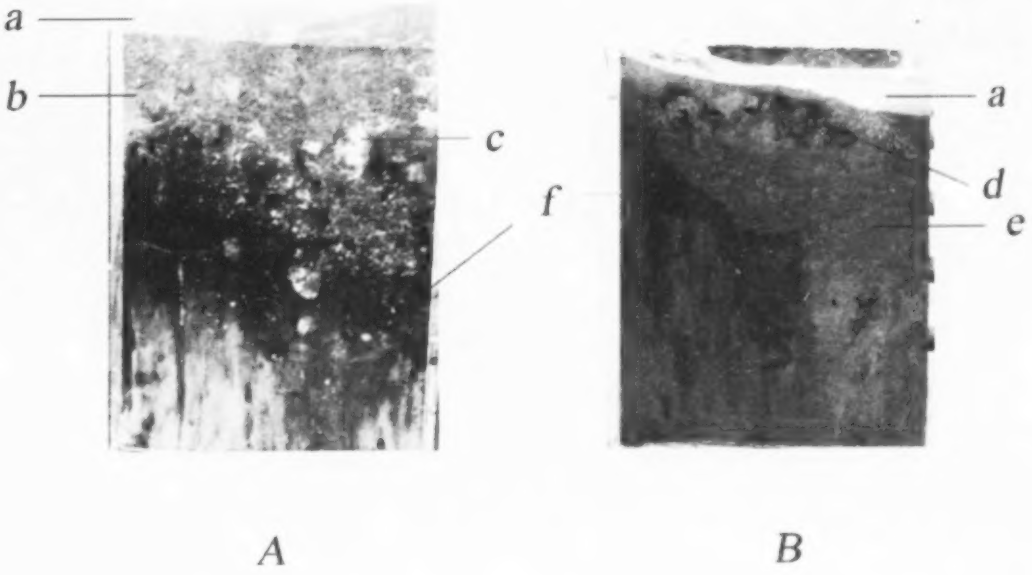


Fig. 5. Sediment profile images from Lime Kiln Bay, Bay of Fundy. A - Farm site; B - Reference site; a - seawater; b - un-decomposed waste feed and feces; c - *Beggiatoa* sp. within un-decomposed wastes; d - feeding voids; e - oxic sediment; f - black sulfide layer.

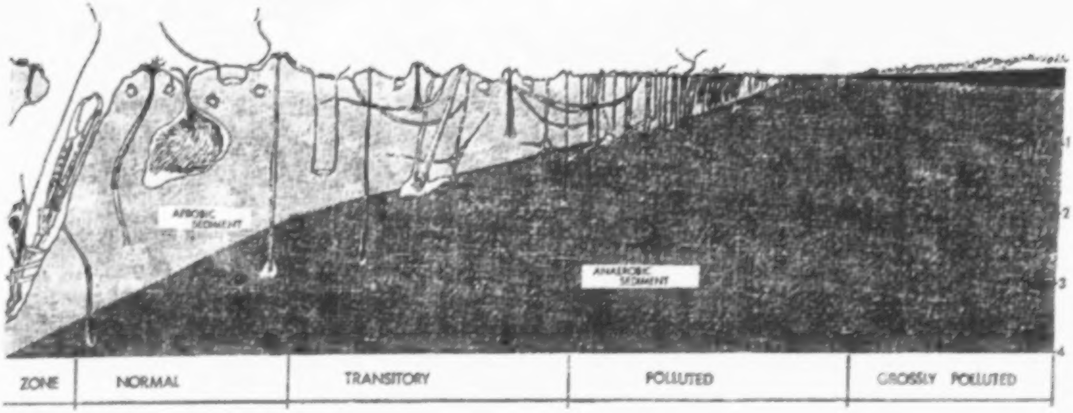

AEROBIC SEDIMENT
ANAEROBIC SEDIMENT
1
2
3
4
ZONE
NORMAL
TRANSITORY
POLLUTED
GROSSLY POLLUTED

Fig. 6. Diagrammatic view of the organic enrichment gradient. The gradient increases from left to right. From Pearson and Rosenberg (1978).

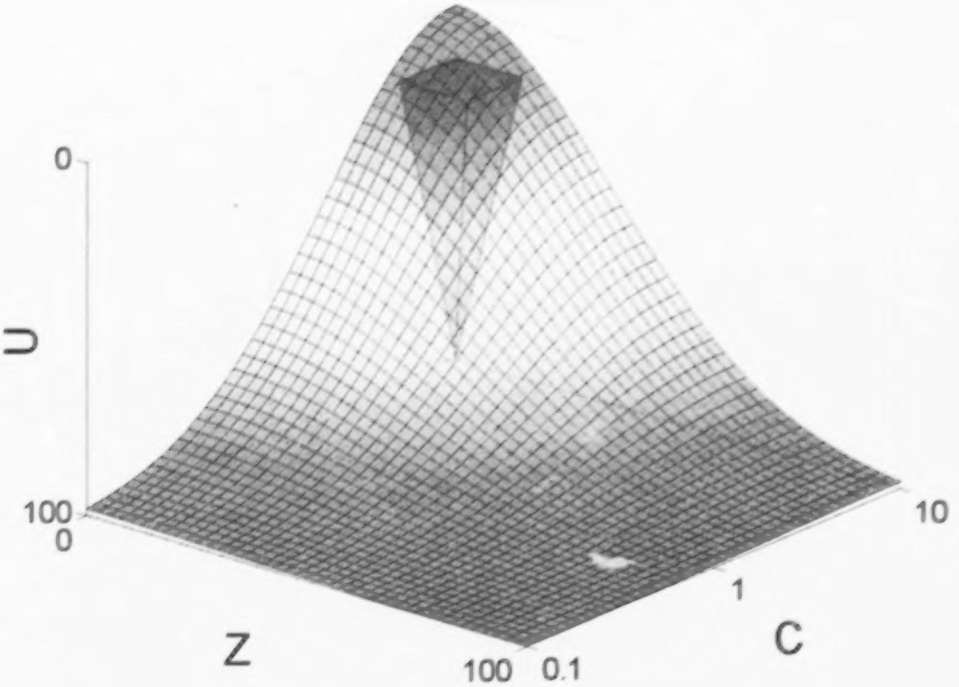


Fig. 7. Three-dimensional diagram showing the dependence of sulfide concentration (to indicate organic enrichment) on the three most important environmental variables which influence it. U = mean velocity (cm s^{-1}); Z = depth (m); and C = sedimentation rate (g C·m^{-2}·d^{-1}).

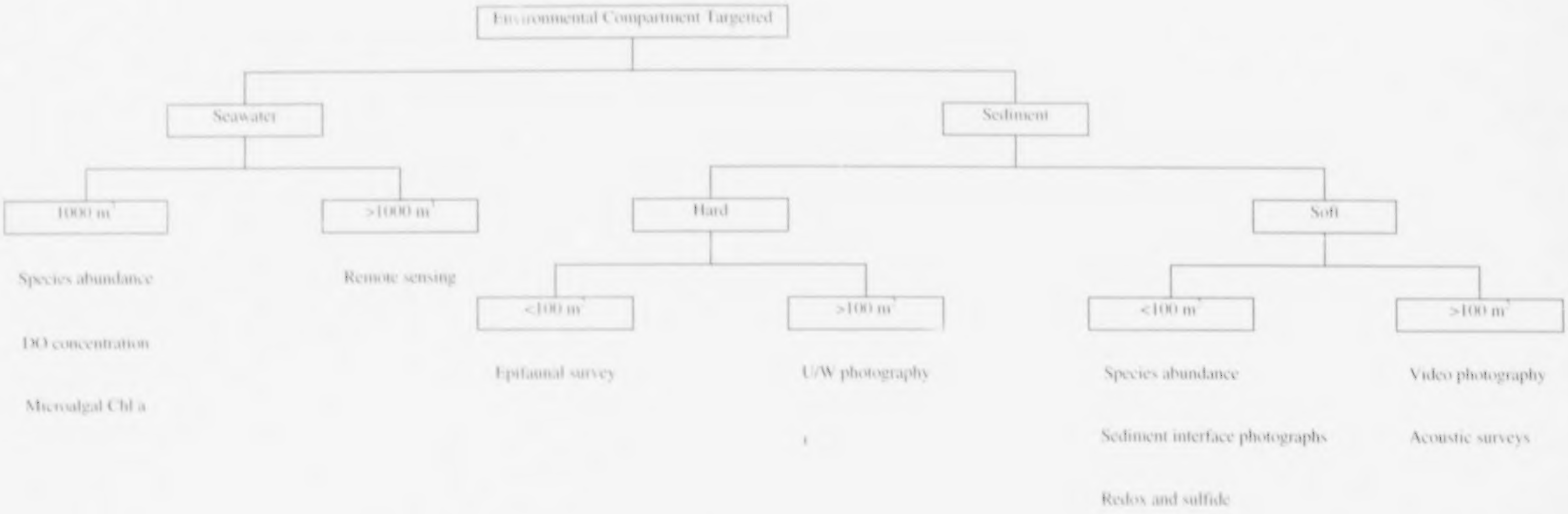

Environmental Compartment Targeted
Seawater
Sediment
1000 m^2
>1000 m^2
Hard
Soft
Species abundance
DO concentration
Microalgal Chl a
Remote sensing
<100 m^2
>100 m^2
<100 m^2
>100 m^2
Epifaunal survey
U/W photography
Species abundance
Sediment interface photographs
Redox and sulfide
Video photography
Acoustic surveys

Fig. 8. Decision tree for choosing appropriate environmental monitoring methods.

ENVIRONMENTAL FATE AND EFFECT OF CHEMICALS ASSOCIATED WITH CANADIAN FRESHWATER AQUACULTURE

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EXECUTIVE SUMMARY

The freshwater aquaculture industry in Canada is growing and with this growth comes the potential use of various chemical agents to treat water, fish or pathogens (e.g. fungicides, disinfectants, anesthetics, pigments, hormones and antibiotics). Despite the broad range of chemicals used in aquaculture around the world, only a subset is licensed for sale in Canada. This review considers chemotherapeutants actively used in Canadian freshwater aquaculture. Sixteen databases were searched for scientific publications on the environmental fate and effect of aquaculture chemotherapeutants. The majority of literature concerns marine systems, with few studies on freshwater aquaculture and only two directly examining freshwater aquaculture in Canada.

There are seven chemicals approved for sale when labeled for food fish use in Canada, including four antibiotic drugs (oxytetracycline, florfenicol, sulfadimethoxine plus ormetoprim, sulfadiazine plus trimethoprim), one anaesthetic (tricaine methanesulphonate) and two fungicides/disinfectants (formaldehyde and hydrogen peroxide) (Health Canada 2001a). Oxolinic acid has been included in this paper, although it is not currently used on Canadian aquaculture farms. However, this chemical is very widely used in salmonid culture outside of Canada, including the United States, and off-label prescription potential exists where veterinarians can legally prescribe it. In addition, oxolinic acid provides a wide degree of information regarding fate and effect data which could be relevant to other antibiotics.

Many studies have been published examining the fate and effect of antibiotics in marine systems, but few have been published with regard to the same issues in freshwater systems. As with all intensive animal husbandry, aquaculture practices create an opportunity for the proliferation and spread of pathogens that can lead to significant mortality of stock and subsequent loss of revenue (Dixon 1994). Antibiotics can be administered directly by injection or by releasing feed containing antibiotics directly into the aquatic ecosystem. Unconsumed medicated feed is available to wild animals. In addition, antibiotic-containing feed can accumulate in the sediments or unabsorbed antibiotics can be released in fish feces or urinary waste (Bjorklund and Bylund 1990, 1991), subsequently influencing the natural bacterial flora, an important component of ecological food webs. Thorpe et al. (1990) estimated that 1.4 to 40.5% of fish feed passed uneaten through an Atlantic salmon sea-cage. However, this may be a conservative estimate since diseased fish feed poorly (Bjorklund et al. 1990), and the majority of active form antibiotic passes unabsorbed through the gastrointestinal tract of fish (Cravedi et al. 1987; Bjorklund and Bylund 1991; Plakas et al. 1998). On the other hand, advances

in feeding technology (e.g. underwater video; Foster et al. 1995) and alternative methods of incorporating antibiotic into feed (Duis et al. 1994) can affect the amount of antibiotic reaching the environment.

Nitrosomonas *spp. and* Nitrobacter *spp. are important bacteria for nutrient cycling in freshwater trophic webs converting ammonia (toxic) to nitrate (non-toxic) (Ricklefs and Miller 2000), but in laboratory microcosms, oxytetracycline greatly inhibited the processing of ammonia (Klaver and Mathews 1994). During disease outbreaks in catfish ponds, the use of antibiotics cured the disease, but reduced bacterial conversion of toxic ammonia to nitrate, allowing ammonia to build up in pond sediments (Klaver and Mathews 1994).*

The evolution of drug resistant strains of pathogenic bacteria is perhaps the most important implication of antibiotic use in aquaculture. Resistance to antibiotics is present in bacterial populations naturally (McPhearson et al. 1991; Johnson and Adams 1992; Spanggaard et al. 1993) and antibiotic use gives resistant strains the opportunity to proliferate and spread. Studies that examined antibiotic resistance following drug therapy at fish farms (Bjorklund et al. 1990, 1991; McPhearson et al. 1991; Nygaard et al. 1992; Samuelsen et al. 1992a; Spanggaard et al. 1993; Ervik et al. 1994; Kerry et al. 1996a; Herwig et al. 1997; Guardabassi et al. 2000) and in microcosms (Kerry et al. 1996; Herwig and Gray 1997; O'Reilly and Smith 2000) show an increased frequency of resistance to several drugs across a variety of bacterial species. However, Kapetanaki et al. (1995) and Vaughan et al. (1996) suggest that increased levels of bacterial drug resistance can arise independently of the presence of a drug (through sterile fish feed, sediments added to microcosm studies, uneaten fish food) and confound studies.

No published studies directly examined the environmental fate and effect of fungicides, disinfectants and anaesthetics within the scope of this review, but several studies have examined tissue deposition, toxicity and stress responses in fish in order to determine appropriate use rates for these chemicals in aquaculture practice (Xu and Rodgers 1993; Howe et al. 1995; Schreier et al. 1996; Rach et al. 1997a, b, 1998; Gaikowski et al. 1998, 1999; Keene et al. 1998; Jung et al. 2001).

In addition to the chemicals discussed above, carotenoid pigments (astaxanthin and canthaxanthin) are added to aquaculture feed to enhance flesh colour in cultured salmonids (Guillou et al. 1995; Metusalach et al. 1997). No studies have been published on the environmental fate and effect of carotenoid pigments introduced in fish feed. Carotenoid pigments could build-up in sediments since the molecules are non-water soluble and stable in the absence of light. Finally, salmonid production can be enhanced by culturing females only, a condition that manipulates the sex phenotype by exposing juvenile fish to 17 -alph-methyltestosterone either through immersion or incorporation in feed. No studies are available on the environmental fate or effect of this hormone within the scope of this review.

KNOWLEDGE GAPS

- *Research is needed on the fate and effect of therapeutants in freshwater systems.*
- *Research is needed to identify the causal factors controlling the distribution, accumulation, and persistence of chemicals in freshwater.*
- *Research is needed regarding the factors affecting microbial resistance to antibiotics in freshwater.*
- *Research is needed to examine the chronic toxicity of antibiotics and other chemotherapeutants to fish and other freshwater organisms.*
- *There is a need to develop standard sampling design and analytical protocols in aquaculture science.*
- *There is a need for an inventory of therapeutant usage patterns that includes reports of what is used, where and in what amount.*

DEVENIR ET EFFETS ENVIRONNEMENTAUX DES PRODUITS CHIMIQUES UTILISÉS EN AQUACULTURE EN EAU DOUCE AU CANADA

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RÉSUMÉ

L'industrie de l'aquaculture en eau douce au Canada est en voie de développement. Et cette expansion peut mener à l'utilisation de divers agents chimiques pour traiter l'eau et les poissons ou lutter contre des agents pathogènes (p. ex. fongicides, désinfectants, anesthésiques, pigments, hormones et antibiotiques). De la vaste gamme de produits chimiques utilisés en aquaculture à l'échelle mondiale, seuls quelques-uns sont homologués au Canada. La présente étude porte sur les agents chimiothérapeutiques activement utilisés en aquaculture en eau douce au Canada. À cette fin, des recherches sur le devenir et les effets environnementaux des agents chimiothérapeutiques utilisés en aquaculture ont été effectuées dans seize bases de publications scientifiques. La majorité des documents publiés portent sur les écosystèmes marins, peu d'études sur l'aquaculture en eau douce ayant été réalisées. Seules deux traitant directement de l'aquaculture en eau douce au Canada ont été localisées.

Au Canada, sept produits chimiques sont homologués pour être utilisés chez les poissons d'élevage destinés à l'alimentation humaine, dont quatre antibiotiques (l'oxytétracycline, le florfénicol, le composé sulfadiméthoxine et ormétoprime, le composé sulfadiazine et triméthoprime), un anesthésique (le méthanesulfonate de tricaïne) et deux fongicides/désinfectants (le formaldéhyde et le peroxyde d'hydrogène) (Santé Canada, 2001a). Bien qu'il ne soit pas utilisé à l'heure actuelle dans les piscicultures canadiennes, l'acide oxolinique est inclus dans le présent document; ce produit chimique étant communément utilisé en salmoniculture à l'extérieur du Canada, y compris aux États-Unis, il peut servir à des utilisations non indiquées sur l'étiquette là où les vétérinaires peuvent légalement le prescrire. En outre, le grand volume de données sur le devenir et les effets de ce composé pourraient se révéler pertinentes pour d'autres antibiotiques.

De nombreuses études ont été publiées sur le devenir et les effets d'antibiotiques dans les écosystèmes marins, mais peu sur les mêmes enjeux dans les écosystèmes d'eau douce. Comme cela est le cas de toutes les formes d'élevage intensif, les pratiques aquacoles offrent aux agents pathogènes la possibilité de proliférer et de se propager, ce qui peut entraîner une forte mortalité chez les stocks d'élevage et la perte conséquente de revenus (Dixon, 1994). Des antibiotiques peuvent être administrés directement par injection des animaux touchés ou par apport d'aliments médicamenteux. Mais les aliments que les poissons d'élevage n'ont pas consommés peuvent l'être par les animaux sauvages. En outre, les aliments contenant des antibiotiques peuvent s'accumuler dans les sédiments ou les antibiotiques non absorbés peuvent être libérés dans le milieu par le biais des

excréments ou de l'urine (Bjorklund et Bylund, 1990, 1991); par la suite, ces antibiotiques ont une incidence sur la flore bactérienne naturelle, un élément important des réseaux alimentaires. Thorpe et al. (1990) ont estimé que de 1,4 à 40,5 % des aliments donnés à du saumon atlantique gardé en cage ne sont pas mangés. Cette estimation peut toutefois être prudente car les poissons malades s'alimentent mal (Bjorklund et al., 1990) et la plus grande partie de la forme active des antibiotiques traverse le tractus gastro-intestinal des poissons sans être absorbée (Cravedi et al., 1987; Bjorklund et Bylund, 1991; Plakas et al., 1998). D'autre part, les progrès réalisés dans les techniques d'alimentation (p. ex. vidéo sous-marine; Foster et al., 1995) et de nouvelles méthodes d'apport d'antibiotiques dans les aliments (Duis et al., 1994) peuvent avoir une incidence sur la quantité d'antibiotiques libérés dans l'environnement.

Les bactéries Nitrosomonas spp. et Nitrobacter spp. jouent un rôle important dans le cycle des substances nutritives dans les chaînes alimentaires en eau douce, convertissant l'ammoniac (toxique) en nitrate (non toxique) (Ricklefs et Miller, 2000), mais dans des microcosmes expérimentaux, l'oxytétracycline a inhibé sensiblement la transformation de l'ammoniac (Klaver et Mathews, 1994). Lors de flambées de cas de maladie dans des étangs d'élevage du poisson-chat, l'utilisation d'antibiotiques a enrayé la maladie, mais a réduit la conversion par les bactéries de l'ammoniac toxique en nitrate, ce qui a mené à son accumulation dans les sédiments des étangs (Klaver et Mathews, 1994).

L'évolution de souches pharmacorésistantes de bactéries pathogènes est peut-être la conséquence la plus importante de l'utilisation d'antibiotiques en aquaculture. Les populations bactériennes ont une résistance naturelle aux antibiotiques (McPhearson et al., 1991; Johnson et Adams, 1992; Spanggaard et al., 1993), et l'utilisation de ceux-ci offre aux souches résistantes la possibilité de proliférer et de se propager. Les résultats d'études sur la résistance aux antibiotiques observée dans des piscicultures (Bjorklund et al., 1990, 1991; McPhearson et al., 1991; Nygaard et al., 1992; Samuelsen et al., 1992a; Spanggaard et al., 1993; Ervik et al., 1994; Kerry et al., 1996a; Herwig et al., 1997; Guardabassi et al., 2000) et des microcosmes (Kerry et al., 1996; Herwig et Gray, 1997; O'Reilly et Smith, 2000) à la suite d'une pharmacothérapie révèlent une fréquence accrue de résistance à plusieurs médicaments chez une panoplie d'espèces bactériennes. Toutefois, Kapetanaki et al. (1995) et Vaughan et al. (1996) suggèrent que les niveaux accrus de résistance des bactéries aux médicaments peuvent se produire indépendamment de la présence d'un médicament (par le biais d'aliments pour poissons stériles, de sédiments ajoutés lors d'études sur le microcosme, des aliments pour poissons non consommés) et brouiller les résultats des études.

Aucune des études publiées évaluées ne portait expressément sur le devenir et les effets environnementaux des fongicides, des désinfectants et des anesthésiques, mais plusieurs examinaient leur distribution dans les tissus, leur toxicité et les réactions de stress chez les poissons en vue de pouvoir établir les taux d'utilisation appropriés de ces produits chimiques en aquaculture (Xu et Rodgers, 1993; Howe et al., 1995; Schreier et al., 1996; Rach et al., 1997a, b, 1998; Gaikowski et al., 1998, 1999; Keene et al., 1998; Jung et al., 2001).

Outre les produits chimiques considérés ci-dessus, des caroténoïdes (l'astaxanthine et la canthaxanthine) sont ajoutés aux aliments pour salmonidés d'élevage afin d'accentuer la couleur de leur chair (Guillou et al.*, 1995; Metusalach* et al.*, 1997). Aucune étude n'a été publiée sur le devenir et les effets de ces pigments. Ils pourraient s'accumuler dans les sédiments étant donné que les molécules ne sont pas hydrosolubles et sont stables en l'absence de lumière. En dernier lieu, on peut accroître la production de salmonidés en n'élevant que des femelles, ce qui se fait en manipulant le sexage génétique : les juvéniles sont exposés à la 17-alpha-méthyltestostérone, soit par immersion dans ce stéroïde ou par apport d'aliments traités. Aucune étude sur le devenir ou les effets environnementaux de cette hormone n'était disponible aux fins d'évaluation dans le cadre du présent examen.*

LACUNES DANS LES CONNAISSANCES

- *Il faut mener des recherches sur le devenir et les effets des agents thérapeutiques dans les écosystèmes d'eau douce.*
- *Il faut mener des recherches en vue d'identifier les facteurs étiologiques qui contrôlent la distribution, l'accumulation et la persistance des produits chimiques dans les eaux douces.*
- *Il faut mener des recherches sur les facteurs ayant une incidence sur la résistance des microbes aux antibiotiques en eau douce.*
- *Il faut mener des recherches sur la toxicité chronique des antibiotiques et d'autres agents chimiothérapeutiques pour les poissons et d'autres organismes dulcicoles.*
- *Il faut élaborer des protocoles normalisés d'échantillonnage et d'analyse en sciences de l'aquaculture.*
- *Il faut faire un inventaire des patrons d'utilisation des agents thérapeutiques, y inclus des rapports sur les produits utilisés, les endroits où ils le sont et les quantités appliquées.*

INTRODUCTION

The aquaculture industry in Canada is growing. As with any intensive husbandry practice, aquaculture operations provide ideal conditions for the rapid proliferation of disease. Chemical agents can be employed to assist fish farmers in preventing and treating such disease outbreaks. In addition, fish producers are under constant competitive pressure to produce high quality products in less time. Food additives, such as pigments and vitamins, are additional chemical agents that can be used to accomplish these demands. With the growth of the aquaculture industry in Canada is potential for use of a wide variety of chemical agents. The impact of these products on the environment is of concern as such contamination may alter the functioning of ecosystems or may pose threats to wildlife. Decisions regarding establishment and operation of aquaculture facilities need to take into account these potential impacts and adjust accordingly. This review is of the fate and effect of chemical agents used in freshwater aquaculture in Canada (Health Canada 2001a; see Tables 1 and 2 for a list of chemicals and their properties). The vast majority of published research on the fate and effect of aquaculture chemotherapeutants is based on marine cage models. Although some freshwater cage-culture operations exist in Canada, the majority of the freshwater aquaculture is land-based hatchery, pond or recirculating systems. As a result, much of the research may not be directly applicable to Canadian freshwater aquaculture. In addition, although the general practices of medicating fish are similar between freshwater and marine cage operations, the differences in the physical and chemical characteristics of the water and bottom substrates make the inferences regarding antibiotic fate in freshwater doubtful.

I will begin with a description of the criteria used to include literature in this review followed by a discussion of chemical agent use in Canadian aquaculture. I will then present, discuss and critique the literature pertaining to the fate and effects of the chemical agents and conclude with a set of recommended research needs.

THE LITERATURE SEARCH

Sixteen databases were searched for primary literature on the environmental fate and effect of aquaculture chemicals (Table 3). The literature search turned-up more than 330 studies examining aquaculture chemicals. However, this list was trimmed to include 121 recent studies (published since 1990) that are reviewed here, based on the following criteria. First, the study had to include at least one of the chemicals listed in Table 1 as 'Approved by Health Canada for Use in Aquaculture.' There is a broad range of chemicals used in aquaculture around the world, but only a subset is approved by the Veterinary Drugs Directorate (VDD; formerly Bureau of Veterinary Drugs) of Health Canada for sale and use in Canadian food fish (Health Canada 2001a). The review is restricted to chemicals that are 'approved for sale.' Bear in mind that 'approved for sale' does not imply 'limited to,' as veterinarians can prescribe drugs 'off-label.' However, veterinarians who do so are responsible for animal safety and for any illegal drug residues that are detected in animal products for human consumption. The release of Emergency Drugs must have VDD authorization before a manufacturer is allowed to sell a limited quantity of unapproved drugs to a licensed veterinarian for the emergency treatment (2004 - http://www.hc-sc.gc.ca/vetdrugs-medsvet/edr_e.html). Second, the study had to address directly the environmental fate and/or effect of one of these chemicals. Third, studies were included if they contained information that could be used to infer or predict the fate or effect of a chemical when no information was presented on the direct fate or effect. For example, I have included in this review many papers on the fate or effect of antibiotics in marine systems and on the fate and effect of oxolinic acid. Whereas these studies have no direct bearing on freshwater systems in Canada (oxolinic acid is not used in Canadian aquaculture), the principles with which they deal (e.g. increased antimicrobial resistance, ingestion of antibiotics by wild fish) are the same between marine and freshwater systems and therefore relevant. Likewise, many investigations directed at finding best method for use of a chemical provided information regarding toxicity to cultured fish. Many of the cultured fish examined in the toxicity studies are present naturally in Canadian freshwater. Finally, studies were included if they provided information on the rate of use or discharge of a chemical listed in Table 1. Such information is useful when attempting to make inferences regarding potential fate and effect.

ANTIBIOTICS

As with all intensive animal husbandry, aquaculture practices create an excellent opportunity for the rapid proliferation and spread of pathogenic bacteria that can lead to significant mortality and subsequent loss of revenue. Hence, fish farmers may use chemicals, such as antibiotics[1] that stop bacteria from growing or kill them outright to maintain an economically viable aquaculture operation (Dixon 1994). Use of antibiotics raises several questions with regard to their fate and effect on local biological systems, some of which are typical of intensive husbandry practices and others are unique to aquaculture. For example, in most husbandry practices, antibiotics are administered to the animals directly under controlled conditions such as inside a barn. However, in aquaculture the most common method of antibiotic delivery is through release of medicated feed directly into the aquatic ecosystem. Direct release of antibiotic-containing feed to the environment means that these chemicals may be available to wild fish either by ingestion or bioconcentration (direct absorption from water through gill membranes) and that they can accumulate and influence the natural bacterial flora, an important component of all ecological food webs. In addition, despite use at trace concentrations at any one point in time, the physico-chemical properties of antibiotics and their metabolites could produce high, localized concentrations over a longer use interval (Halling-Sorensen et al. 1998).

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[1] Strictly, the term antibiotic refers to molecules that will affect either bacteria (antibacterial) or fungi (antifungal) (Walsh 2003) and occur naturally (Shnayerson and Plotkin 2002). However, antibiotic is used interchangeably with antibacterial throughout the literature. Here, I use antibiotic to refer to an agent that is antibacterial. I use antibiotic in reference to synthetic drugs as well.

THE DRUGS

Four antibiotics are approved by Health Canada (2001a) for use in aquaculture: oxytetracycline hydrochloride, florfenicol, sulfadiazine + trimethoprim, and sulfadimethoxine + ormetoprim (Table 1; Health Canada 2001a).

Oxytetracycline is a naturally occurring antibiotic produced by *Streptomyces* fungi (Bjorklund 1991; Johnson and Adams 1992). It is effective against a broad spectrum of microorganisms, including both gram negative and gram positive bacteria, by binding to rRNA and inhibiting protein synthesis (Bjorklund 1991; Johnson and Adams 1992; Stoffregen et al. 1996). Oxytetracycline was first used in aquaculture in 1951 against ulcer disease (*Haemophilus piscium*) in brook charr (*Salvelinus fontinalis*; Bjorklund 1991), but its use has expanded to include treatment of a wide variety of bacterial fish diseases, including furunculosis (*Aeromonas salmonicida*), septicaemia (*A. hydrophila*), vibriosis (*Vibrio anguillarum*) and cold-water disease (*Flavobacterium psychrophilum*; Bjorklund 1991; Stoffregen et al. 1996). In Canada, oxytetracycline is available 'over the counter' at label dosage (75 $mg \cdot kg^{-1}$ $fish \cdot day^{-1}$ for oral administration of the drug for 1 to 10 days) (Health Canada 2001b), but requires prescription for dosage above 75 $mg \cdot kg^{-1}$. Oxytetracycline is the most widely used antibiotic in aquaculture around the world (Bjorklund 1991) and in Canada (Sheppard 2000), and as such, much of the research on the environmental fate and effect of antibiotics has been focussed on this drug.

Florfenicol is a synthetic antibiotic effective in the treatment of furunculosis (*A. salmonicida*) and vibriosis (*V. anguillarum*). Its method of effect is through binding to ribosomes and preventing protein synthesis (Lunden et al. 1999). Recommended dosage rate is 10 $mg \cdot kg^{-1}$ $fish \cdot day^{-1}$ for 10 days (Aquaflor Technical Monograph, Schering-Plough Animal Health).

Both sulfadiazine + trimethoprim and sulfadimethoxine + ormetoprim are potentiated sulfonamides (Stoffregen et al. 1996). Potentiated sulfonamides also have broad spectrum antimicrobial capabilities and have been used to treat diseases associated with *A. salmonicida*, *A. hydrophila*, *V. anguillarum*, *V. salmonicida*, and *Yersinia ruckeri* isolated from rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) (Stoffregen et al. 1996) and *Edwardsiella ictalluri* in channel catfish (*Ictaluris punctatus*) (Johnson et al. 1993; Starliper et al. 1993). Typical sulfamethoxine + ormetoprim dosage is roughly 50 $mg \cdot kg^{-1}$ $fish \cdot day^{-1}$ (Johnson et al. 1993; Starliper et al. 1993; Treves-Brown 2000), and sulfadiazine + trimethoprim dosage is 30 $mg \cdot kg^{-1}$ $fish \cdot day^{-1}$ for 7 to 10 days (Treves-Brown 2000).

USE OF ANTIBIOTICS IN CANADIAN FRESHWATER AQUACULTURE

Overall, the vast majority of primary literature examining the fate and impact of antibiotics in the environment concerns marine systems, with very few studies conducted on freshwater aquaculture and only two directly examining freshwater aquaculture in Canada. Thorburn and Moccia (1993) conducted a personal interview survey during

1990-1991 to determine why and how trout farmers in Ontario were using chemotherapeutants. (Loosely, a chemotherapeutant is any chemical used prophylactically or therapeutically to treat disease.) They found that, although 26% of farmers (n=62) felt that disease had important economic consequences for production, many Ontario farmers use therapeutants only rarely. However, only a few farms were completely therapeutant-free and a few farms used chemotherapeutants frequently. A subsequent study (Thorburn et al. 2001) showed that therapeutant use is lower for faster growing and larger fish than for slower growing and smaller fish.

Assuming freshwater aquaculture practices are similar across Canada, the results of Thorburn and Moccia's (1993) survey indicate that chemotherapeutants are used by the aquaculture industry and that information is needed on the environmental fate and effects of chemotherapeutants in Canadian freshwater aquaculture. However, as the authors point out, there are potential problems with their survey. Some antibiotics may not have been reported in the survey.

Detailed estimates for the use of antimicrobials in the Canadian agrifood sector are not available (Conly 2002). However, the Canadian Food Inspection Agency has collected data regarding antibiotics addition during feed manufacture (Health Canada 2001b) but does not have statistics regarding 'over the counter' drug sales. One province, British Columbia, has records regarding antibiotic use in aquaculture within that province. However, the vast majority of antibiotic (roughly 11 000 kg in 2001[2]) is used in marine aquaculture. There is a need for an inventory of usage patterns in Canadian freshwater aquaculture that includes reports of what is used, where and in what amount.

ENVIRONMENTAL FATE OF ANTIBIOTICS USED IN AQUACULTURE

Feeding and Absorption Efficiency

The majority of antibiotic treatment for finfish aquaculture is through incorporation with feed. There are three paths in which antibiotics can get into the environment. Medicated feed presented to fish may not be eaten and end up on the bottom, the antibiotic may leach from the feed before the feed is eaten or reaches the bottom, or the feed may be eaten but unabsorbed antibiotic released in feces or urinary waste (Bjorklund and Bylund 1990; Jones et al. 2002). Bjorklund and Bylund (1991) determined that only 5.6% of the oral dose of oxytetracycline (75 $mg \cdot kg^{-1}$) and 13.6% of the oral dose of oxolinic acid (75 $mg \cdot kg^{-1}$) was absorbed by rainbow trout. Cravedi et al. (1987) showed that rainbow trout absorbed only 7.1 to 8.6% of oral oxytetracycline (20-100 $mg \cdot kg^{-1}$) mixed with feed. Plakas et al. (1998) found that channel catfish absorbed only 1 to 1.7% of an oral dose a tetracycline mixed in feed (4 and 80 $mg \cdot kg^{-1}$). These findings suggest that the majority of antibiotic treatment passes unabsorbed through the gastrointestinal tract of fish and reaches the environment in active form (Bjorklund and Bylund 1991). Thorpe et al. (1990) estimated that 1.4 to 40.5% of feed passed uneaten through an Atlantic salmon sea-cage. Because diseased fish feed poorly (Bjorklund et al. 1990), the range reported by

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[2] Medicated Feed Statistics, Ministry of Agriculture, Food and Fisheries, Government of British Columbia: http://www.agf.gov.bc.ca/fisheries /health/antibiotics.htm [accessed 5 June 2003].

Thorpe et al. (1990) may be a conservative estimate of the proportion of medicated feed reaching the sediments of pond or cage culture facilities.

Although feeding efficiency impacts how much feed, and therefore antibiotic, reaches sediment, farmer feed presentation behaviour probably plays a larger role. For example, Coyne et al. (1994) observed very little feed reaching the bottom under a cage culture facility in Ireland, whereas Samuelsen et al. (1992a) observed a thick organic sludge under a farm in Norway. Recently, acoustic sensors, infrared sensors and underwater video systems have been used to monitor excess feed passing through sea-cages (e.g. Juell et al. 1993; Ervik et al. 1994; Foster et al. 1995; Madrid et al. 1997). These techniques are all effective in adjusting feed inputs to fish feeding behaviour and subsequently reducing the amount of feed (and antibiotic) that reaches the sediment.

The type of antibiotic and the method of antibiotic incorporation into feed pose additional issues regarding how much antibiotic reaches the environment. For example, Duis et al. (1994) found that following 3 seconds of immersion in water, 8.3% of trimethoprim, 4.5% of oxytetracycline and 0% of oxolinic acid had leached from medicated feed pellets prepared by oil-coating. On the other hand, only 1%, 3.9% and 0% of trimethoprim, oxytetracycline and oxolinic acid had leached from pellets prepared by alginate coating. By 3 min, oil coated pellets had lost 19.8%, 15.3% and 2.5% of trimethoprim, oxytetracycline and oxolinic acid respectively, whereas alginate prepared pellets had only lost 8.3%, 4.5% and 0% respectively. Finally, at 15 min, oil-coated pellets had lost 67.3%, 50.7% and 8.4% of trimethoprim, oxytetracycline and oxolinic acid respectively, and alginate treated pellets had lost 6.3%, 24.1% and 0% respectively.

Rigos et al. (1999) found that oxytetracycline was lost to surrounding water more rapidly when incorporated with the feed by oil coating than when mixed directly with the feed by the manufacturer and that the loss of antibiotic from the feed was greater at higher water temperatures. The different leaching rates for medicated feed preparation types examined by Duis et al. (1994) and Rigos et al. (1999) pose different sets of issues for aquaculturists using pond culture versus those using open-water cage culture. For example, in pond culture, sediments can be removed to prevent contaminants from reaching the natural environment (Bebak-Williams et al. 2002). Therefore, having medicated feed reach the sediments and be trapped with most of the antibiotic still incorporated may be advantageous to having the antibiotic leach into the water rapidly and released into adjacent streams. On the other hand, in cage culture, having medication leach from the feed rapidly and diluted by large quantities of water may be preferred to having the medication remain in the feed longer and be consumed by wild fish or reach the sediments under a cage.

Stoffregen et al. (1996) reported that as little as 0.6% of oxytetracycline is absorbed by carp through oral dosing. In addition, Bjorklund et al. (1990) found only 2 $\mu g \cdot g^{-1}$ in rainbow trout muscle following 10 days of treatment at 100 mg oxytetracycline$\cdot kg^{-1}$ of fish. Bjorklund and Bylund (1990) and Rogstad et al. (1991) showed that the overall absorption rate and accumulation of oxytetracycline in rainbow trout is very low but variable depending upon tissue type and ambient temperature. For example, Rogstad et al. (1991) found that a 150 mg·kg^{-1} oral dose (placed directly into the stomach) reached peak concentrations in stomach tissue at 24 h (1180 µg·g^{-1}), mucus at 48 h (19.1 µg·g^{-1}), liver and skin at 72 h (44.7 µg·g^{-1} and 7.14 µg·g^{-1} respectively) and plasma and muscle at 75 to 80 h (3.2 µg·g^{-1} and 1.7 µg·g^{-1} respectively). Vertebral concentrations continued to increase throughout the experiment (to 336 h). Overall, this rate of absorption is slow (taking greater than 72 h to reach appreciable levels in muscle and liver tissue) and inefficient. Less than 1% had been absorbed by 24 h, and overall absorption was estimated to be 2.6% at 72 h. Unabsorbed oxytetracycline was excreted quickly, whereas that which was absorbed was excreted slowly over a 2-week period.

Bjorklund and Bylund (1990) found that a 75 mg·kg^{-1} oral dose of oxytetracycline administered (via feeding tube) to rainbow trout held at 16ºC reached peak liver concentration 1 h post administration (2.1 µg·mL^{-1}), whereas fish held at 10ºC and 5ºC reached peak concentrations of 5.3 µg·mL^{-1} at 12 h and 3.2 µg·mL^{-1} at 24 h respectively. The results for muscle tissue followed the same pattern as that for liver tissue, but were delayed by 48 h. Elimination rates were also highest at higher temperatures. Drug concentrations declined below detectable limits (<0.01 µg·g^{-1}) within 20 days for fish held at 16ºC, 30 days for fish held at 10ºC and was still detectable at 0.1 µg·mL^{-1} at 40 days for fish held at 5ºC. In a subsequent study, Bjorklund and Bylund (1991) estimated that 94.4% of 75 µg·g^{-1} oral doses of oxytetracycline passed through the gut of juvenile rainbow trout to the environment in active form with only 5.6% being absorbed. Elema et al. (1996) found that the only 2.0% of oxytetracycline was absorbed by Atlantic salmon in saltwater. The results of these studies suggest that the bioavailability of this drug is slightly higher in freshwater than in marine systems. However, the freshwater (Bjorklund and Bylund 1990, 1991) and seawater (Elema et al. 1996) studies were conducted on different species, so inferences regarding the effects of salinity on bioavailability based on these studies are limited. Oxolinic acid and flumequin are absorbed more readily by rainbow trout and Atlantic salmon (respectively) in freshwater than in saltwater (Ishida 1992; Sohlberg et al. 2002). Additionally, the drugs were eliminated much more slowly over longer time intervals by fish held in freshwater.

Haug and Hals (2000) compared the absorption of oxytetracycline by Arctic charr (*Salvelinus alpinus*) fed diets with different drug incorporation techniques. Oxytetracycline was incorporated with feed using either liposome/alginate or agar and fish fed at a rate of 100 mg oxytetracycline·kg^{-1} fish. They found that the liposome/alginate method reduced the bioavailability of oxytetracycline relative to the agar method (3.2% compared to 7.3% respectively). Both methods appear to increase the bioavailability of oxytetracycline relative to previous studies, but again, this inference is not strong because in addition to using different drug/feed preparations, the study uses a completely different species of fish.

Regardless, excreted oxytetracycline may represent a significant, longer-term exposure risk. Bebak-Williams et al. (2002) found that oxytetracycline was present at 22 µg·g^{-1} sediment, even after replacing the sediments in recirculating tanks at the end of 10 days of therapy. Presumably, the source of the oxytetracycline in the new sediment was excretion by the fish.

Whereas the bioavailability of oxytetracycline is generally low, that of florfenicol is high at 96.5% (Atlantic salmon under experimental seawater conditions; Martinsen et al. 1993). In addition, florfenicol is eliminated from tissues rapidly (elimination half-life of about 12 h compared to 60.3 h for oxytetracycline), so unlike oxytetracycline, release of florfenicol to the environment is over a short time period. Nordmo et al. (1998) suggest that the high bioavailability of florfenicol means lower doses are required to treat disease outbreaks and less will be released to the environment as a consequence.

Antibiotics can bioconcentrate (move directly from water to tissue) whether released directly to the water from feed or excreted by medicated fish. Jones et al. (2002) calculated the bioconcentration factor (the ratio of chemical concentration in the organism to that in surrounding water) for oxytetracycline in freshwater cyanobacteria and freshwater algae to be roughly 3.2. The food web implications of bioconcentration near freshwater aquaculture operations are not known but certainly would be dependent on the concentration of an antibiotic in the surrounding water.

Ingestion by Wild Fish

Aquaculture cages attract wild fish (Dempster et al. 2002). Feed can end up outside of the cages and available to wild fish (Thorpe et al. 1990). Carss (1990) found that 30 to 73% of wild rainbow trout caught near freshwater cage culture facilities in Scotland had commercial feed in their guts. The proportion of wild fish that had feed pellets in their gut varied depending on season, with the lowest occurrence in August (30.8%) and the highest in October (73.3%). Bjorklund et al. (1990) examined oxytetracycline in wild fish near two cage culture operations in the Baltic Sea. Both farms, one culturing rainbow trout and one Atlantic salmon, applied oxytetracycline (100 $mg \cdot kg^{-1}$ per day orally) for 10 days. HPLC analysis of tissues from bleak (*Alburnus alburnus*) and roach (*Rutilus rutilus*) caught near the cages revealed that bleak at one facility had from 0.2 to 1.3 $\mu g \cdot g^{-1}$ muscle tissue and roach at the other had 0.05 to 0.1 $\mu g \cdot g^{-1}$, values which are above those set by Health Canada in edible tissues (Table 2). Unfortunately, no control site was used to compare the near cage oxytetracycline levels with those attributable to other sources. In a subsequent study, Bjorklund et al. (1991) examined oxolinic acid residues in bleak at one fish farm in the Baltic Sea receiving 10 $mg \cdot kg^{-1}$ per day for 10 days. Although oxolinic acid was detected in the cultured fish, it was not detected in the sediment or in any of the 24 wild fish examined, suggesting that the wild fish had not ingested any of the medicated feed.

Samuelsen et al. (1992b) examined the occurrence of oxolinic acid in several species of wild fish near two Norwegian marine fish farms receiving 25 $mg \cdot kg^{-1}$ per day for 10 days (Farm 1) and 54 $mg \cdot kg^{-1}$ per day for 8 days (Farm 2). Most (91.8%, n=61) fish sampled at the fish farms on the day treatment ended had detectable concentrations (>0.01 $\mu g \cdot g^{-1}$) of oxolinic acid in muscle tissue. All (n=42) of the fish at Farm 1 had detectable concentrations of oxolinic acid in muscle tissue. The average concentration across all species at Farm 1 was 4.38 μg·g^{-1} on the day treatment terminated, with the highest concentration reaching 12.51 μg·g^{-1}. Oxolinic acid residues were detectable in fish caught 400 m away from the cage site at a depth of 100 m and were still detectable in muscle tissues of several species 13 days after treatment had been terminated.

Ervik et al. (1994) examined oxolinic acid concentrations in muscle tissue of several species near six marine farms along the west coast of Norway. HPLC showed that fish from all farms had detectable levels (>0.01 μg·g^{-1}) of oxolinic acid. Occurrence of detectable levels of oxolinic acid in fish sampled near the various farms range from 72 to 100%. All fish sampled at one farm site (n=32 fish representing five species) had detectable concentrations of oxolinic acid. Saithe (*Pollachius virens*) and Haddock (*Melanorammus aeglefinus*) had the highest mean muscle concentrations of oxolinic acid at 5.56 and 5.02 μg·g^{-1} respectively.

Ingestion by Invertebrates

LeBris et al. (1995) examined the tissue concentrations of oxytetracycline in three species of shellfish (*Crassostrea gigas, Ruditapes philippinarum and Scrobicularia plana*) in an experimental microcosm. Four 2 X 2 m tanks received random assemblages of the three species. One tank was a control and received seawater, whereas the other three tanks received effluent from a fifth tank that simulated a fish farm being treated with oxytetracycline for seven days. Samples were drawn from each tank during the seven day oxytetracycline phase and for 14 days post-oxytetracycline treatment. Oxytetracycline levels increased from 0 μg·g^{-1} in all three species at the start of the experiment to a maximum of 1.3 μg·g^{-1} in *C. gigas* by Day 7, 0.5 μg·g^{-1} in *R. philippinarum* by Day 2 and 1.0 μg·g^{-1} in *S. plana* by Day 4. Levels of oxytetracycline declined gradually once oxytetracycline treatment ceased but were still detectable in all three species at the end of the experiment. No oxytetracycline residues were detected in any of the shellfish from the control tank.

Capone et al. (1996) sampled tissues from three species of invertebrates (oyster, *Crasosteria gigas*, Dungeness crab, *Cancer magister* and red rock crab, *Cancer productus*) around an Atlantic salmon cage facility in Puget Sound, Washington. The salmon had recently undergone treatment with oxytetracycline (186 kg over approximately 17 days). Samples from each species were collected in June, July, August, September and October. Oxytetracycline residues were never found in oysters, and trace amounts were found in Dungeness crab in June, July and October. Red rock crab contained trace amounts in June and August, and none in October. Oxytetracycline residues in red rock crab ranged from trace to >3.8 μg·g^{-1} during July and August. Although all three species were collected from a farm location, oxytetracycline was found only in the two detritivores (the crabs) but not the planktivore (the oyster).

Samuelsen et al. (1992b) examined oxolinic acid residues in blue mussel (*Mytilus edulis*) and crab (*Cancer pagurus*) near the Norwegian fish farms mentioned in the previous section. Oxolinic acid residues were present in both species at both farms, although they were higher at Farm 1 than at Farm 2. For example, average oxolinic acid concentration in blue mussels was 0.65 $\mu g \cdot g^{-1}$ at Farm 1 and 0.07 $\mu g \cdot g^{-1}$ at Farm 2 and in crabs was 0.81 $\mu g \cdot g^{-1}$ at Farm 1 and 0.01 $\mu g \cdot g^{-1}$ at Farm 2 on the day antibiotic therapy finished. Blue mussels contained no oxolinic acid after the first sampling time. On the other hand, oxolinic acid was detected in crab throughout the 2-week post-treatment sampling period, although levels did drop to just above detectable by Day 13.

Accumulation and Persistence of Antibiotics in Fish Farm Sediments

Studies examining the accumulation and persistence of antibiotic residues in fish farm sediments have employed three different methodologies. First, many authors take advantage of natural experiments provided by routine activity at aquaculture facilities. Typically in this type of study, a researcher samples the sediments under and adjacent to a fish farm before, during and after application of known amounts of antibiotics. The second style is in the form of laboratory microcosm experiments in which researchers set-up controlled and standardized systems containing natural sediments, and inputs of feed and antibiotic that simulate a real fish farm. Accumulation and degradation of residues are monitored over time. The third type is a hybrid between the first two in which researchers place known quantities of sediment containing known amounts of antibiotic in natural systems and monitor the sediment samples periodically over time.

Field Surveys: Smith et al. (1994a) compared oxytetracycline in the effluent of a freshwater fish farm in Ireland comprised of 12 X 12 m and 7 X 20 m ponds. Unfiltered effluent and water from an experimental filtration system were collected following oxytetracycline therapy during the morning feed and following an afternoon feed with unmedicated feed. Oxytetracycline was never detected in the untreated effluent, but was detected in the filtrate. The authors suggest that the non-detectable results cannot be taken to indicate that the hatchery is discharging negligible amounts of the antibiotic. Water flow through the farm is 40 million L per day, and the hatchery could discharge up to 800 g of oxytetracycline in the untreated effluent without any being detected with an assay at a lower detection limit of 0.02 $\mu g \cdot mL^{-1}$. A second observation made by the researchers was that peak daily oxytetracycline concentrations occurred following the afternoon unmedicated feed, not after the morning medicated feed as had been expected. This observation suggests that the majority of oxytetracycline feed had been ingested during the morning feed and unabsorbed oxytetracycline was displaced during the afternoon feed. The use of the filter clearly showed release of oxytetracycline from the fish farm. Timing of the release of oxytetracycline observed in this study may not be typical of oxytetracycline therapy because the fish receiving medicated feed in this study were healthy. Therapy of diseased fish may show higher levels of oxytetracycline following morning feeds because they feed less efficiently than healthy ones.

Bjorklund et al. (1990) examined accumulation and persistence of oxytetracycline in sediments near cage culture operations in the Baltic Sea along the southwest coast of Finland. Two farms, one culturing rainbow trout and the other Atlantic salmon, underwent 10 days of oxytetracycline therapy at 100 $mg \cdot kg^{-1}$. Sediment samples were collected at each location periodically over the course of 308 days (five from Farm A and four from Farm B). The sediments under the cage sites were anoxic and comprised of considerable amounts of organic material (5-10 cm under Farm A and 10-30 cm under Farm B). Oxytetracycline was present in the sediment at 0.1 μg·g^{-1} and 2.0 μg·g^{-1} at Farm A and B respectively one day following cessation of therapy. Oxytetracycline was not detected in the sediments under Farm A after seven days post-therapy. However, oxytetracycline concentration peaked at 16.0 μg·g^{-1} at Day 8 post-therapy and was still detectable at 2.0 μg·g^{-1} on Day 308 post-therapy at Farm B. There were several differences between Farm A and B, but current seemed to be the most obvious. Farm A was located in an area of high current and Farm B in an area of low current. The authors attributed the difference in both organic matter and oxytetracycline levels to differences in water current between the two farm sites. They concluded that reduction of oxytetracycline levels in anoxic fish farm sediments (Farm A) was mainly due to leakage and washing away with currents and only secondarily due to decomposition of the drug. Unfortunately, chemical characteristics of the sediment at both farms were not measured, making it difficult to confirm that current alone was responsible for the different persistence times.

A subsequent study by Bjorklund et al. (1991) compared sediment levels of oxytetracycline and oxolinic acid beneath five rainbow trout farms in brackish water (0.6% salinity). Dosage of oxytetracycline was 6 to 11 times greater than for oxolinic acid. However, oxytetracycline was present in the sediments at concentrations roughly 30 to 40 times greater than for oxolinic acid. In addition, oxolinic acid was not detectable in the sediment six days post-treatment, but oxytetracycline was still present 12 days post-treatment (1-4 μg·g^{-1} depending on the farm). The authors also noted that sediments under the cages were overlain by anoxic organic sludge.

Samuelsen et al. (1992a) conducted a similar study near Bergen, Norway. However, sediments were monitored under three cages at a single farm. In addition, the cages received different amounts of oxytetracycline. One cage was used as a control cage in which no oxytetracycline therapy was administered. In a second cage, fish were treated with 75 mg·kg^{-1} per day of oxytetracycline for 10 days; and in a third cage, fish were treated with the 10-day mg·kg^{-1} per day dose, plus at Day 51 post initial therapy these fish were treated again for five days. Once again, the sediments under the cages were thick with anoxic, organic sediments. Initial (one day post-therapy) oxytetracycline levels were highest under the cage receiving two treatments (300 ppm), intermediate under the cage receiving one treatment (200 ppm) and lowest under the cage receiving no treatments (25 ppm). Oxytetracycline concentration in the sediment under all three cages decreased gradually for roughly 250 days, after which it leveled-off and was still detectable 550 days post-therapy.

Coyne et al. (1994) examined oxytetracycline residues in sediments under two sets of cages in Galway Bay, Ireland. One set, Block 6, received 10 days of oxytetracycline therapy at 125 mg·kg^{-1} (865 g·day^{-1}) and the other, Block 7, received 12 days therapy at 125 mg·kg^{-1} (865 g·day^{-1}). Sediment samples were collected at one location under a cage at Block 6 and at 18 locations (seven directly under cages and 11 beyond the edge of the cages to the north, south, east and west) along two orthogonal transects passing through

Block 7. Oxytetracycline was detected in the sediments at Block 6 within three days of the end of treatment at 9.9 $\mu g \cdot g^{-1}$, and fell exponentially to 2.3 $\mu g \cdot g^{-1}$ by 32 days post-treatment. Only 1.6 $\mu g \cdot g^{-1}$ was present by Day 66 post-treatment. At Block 7, oxytetracycline was present in the all sediment samples taken directly under the cages (mean= 0.9 $\mu g \cdot g^{-1}$), and at locations sampled beyond the edge of the cages 25 m to the east and south and 25 to 50 m to the west. By Day 19 post-treatment, oxytetracycline was detectable in only five of the under-cage samples (mean=3.3 $\mu g \cdot g^{-1}$) and below detectable limits at all locations beyond the edge of the cages. By Day 32 post-treatment, mean oxytetracycline concentration under the cages had fallen to 1.6 $\mu g \cdot g^{-1}$ and was not detected beyond the cage edges, and by Day 77 was no longer detectable anywhere at the facility.

In contrast to the studies by Bjorklund et al. (1990) and Samuelsen et al. (1992a), the study by Coyne et al. (1994) observed very little organic sludge under the cages. In addition, the levels of oxytetracycline in the sediments were lower than observed in either Bjorklund et al. (1990) or Samuelsen et al. (1992a). Coyne et al. (1994) suggest that feeding regimes probably play a large role in the amount of antibiotic deposited in sediments under cages. However, Coyne et al. (1994) observed that sediment distribution around the cages they studied was predictable based on current models. In addition, Bjorklund et al. (1990) felt that the variation in organic deposits and oxytetracycline observed between the two farms was due to different amounts of current between the two sites.

Capone et al. (1996) examined accumulation and persistence of oxytetracycline and sulfamethoxine + ormetoprim in sediments under three Atlantic salmon farms in Puget Sound. These farms differed in their pattern of antibacterial use. For example, total antibacterial use for the year previous to the study varied from 224 kg at Farm A, 18 kg at Farm B and 8.5 kg at Farm C. Total oxytetracycline use at these farms followed the same pattern, 186 kg, 9.5 kg and 3.0 kg for Farm A, B and C respectively. Total amounts of sulfamethoxine + ormetoprim used and the rate of use at each farm were not reported. Sediment samples at each farm were collected at monthly intervals throughout the year from sites directly under the cages and at 0, 30, 100 and 200 m downshore (based on prevailing current patterns) along transects that followed the isobath. Collection of sediment samples was timed at each farm to coincide with before, during and after antibiotic therapy. Oxytetracycline was present in the sediments (0.2-1.0 $\mu g \cdot g^{-1}$) 30 days prior to therapy in half of the samples collected under Farm A. Initial post-treatment levels of oxytetracycline were highest under Farm A (1.0-4.0 $\mu g \cdot g^{-1}$) and they remained high for 44 days post-treatment. Oxytetracycline in the sediments had dropped to 1.0 $\mu g \cdot g^{-1}$ by Day 77 post-treatment. In addition, oxytetracycline was detected in half of the samples collected 30 m beyond Farm A for the June sample, but never at 100 and 200 m from the farm nor at the 30 m sample at any other sample time. Highest concentration of oxytetracycline at Farm B was 0.2 to 1.0 $\mu g \cdot g^{-1}$ directly under the cages immediately after antibiotic therapy. Oxytetracycline was detectable in half the samples collected under the farm at 30 days post-treatment, but not after that, and never detected at samples collected beyond the edge of the farm. Oxytetracycline was not detected in the sediments under Farm C.

Hektoen et al. (1995) examined the persistence of sulfadiazine + trimethoprim, oxytetracycline, florfenicol and oxolinic acid in boxes of sediment placed at 15 m depth for six months along the coast of Norway. Florfenicol had the shortest half-life (Table 4), decreasing from 50 $\mu g \cdot g^{-1}$ sediment to less than 5 $\mu g \cdot g^{-1}$ sediment by 10 days. Some of the florfenicol was metabolized to florfenicol amine, but the loss of the majority of the drug was a result of other causes, as florfenicol amine was present maximally at 1.2 $\mu g \cdot g^{-1}$. Sulfadiazine had the next shortest half-life (Table 4), decreasing from 5000 to 500 $ng \cdot g^{-1}$ in 180 days; and tremethoprim had the next shortest half-life, decreasing from 1000 to 100 $ng \cdot g^{-1}$ in 180 days. Oxytetracycline and oxolinic acid had the longest half-lives, decreasing from 200 to 60 $\mu g \cdot g^{-1}$ and 100 to 10 $\mu g \cdot g^{-1}$ over 225 days for each respectively.

Laboratory Studies: Laboratory studies of antimicrobial drugs in sediments are principally designed to determine what factors influence persistence and how persistence differs among various drugs. Bakal and Stoskopf (2001) found that the concentration of sulfamethoxine + ormetoprim in 600-mL beakers was unaffected by salinity or pH over 365 days. Sulfamethoxine concentration at 4°C was half that of the same compound at 25°C. However, when the 4°C beaker was brought to 25°C, the concentration of sulfamethoxine increased to values similar to the beaker that had been held at 25°C continuously. Ormetoprim was unaffected by temperature, but its concentration was reduced in the presence of bentonite clay relative to other potential aquaculture pond liners (e.g. PVC, silica sand or polyolifin). Despite the relative logistical ease of this study (recognized by the authors), there was no attempt to replicate. Only one beaker was used per treatment combination and three replicates were measured per beaker.

Doi and Stoskopf (2000) conducted a similar experiment to examine persistence of oxytetracycline in relation to several variables (temperature, pH, light, presence of clay, presence of organic matter). They found that oxytetracycline degraded rapidly over 21 days in 600-mL beakers held at 43°C but slowly at 4 and 25°C. They also found that oxytetracycline was more stable at pH 3 than at pH 7 and pH 10 and that it was more stable in the absence of light. Clay was observed to reduce the availability of the drug and there was no additional effect of dead organic matter on the impact of clay. The authors did not attempt to examine the potential interaction among the variables: the effect of each variable on oxytetracycline was examined separately. Therefore, inferences regarding the additive or multiplicative effects of the variables are not possible. This study also lacked appropriate replication as with the previous study.

Whereas Doi and Stoskopf (2000) found no effect of organic matter on oxytetracycline, Holten Lützhøft et al. (2000) found that dissolved organic carbon bound to oxolinic acid reducing its availability. However, such binding in nature, they argue, will make the compound unavailable for degradation and may facilitate its accumulation.

Bjorklund et al. (1991) compared degradation rates of oxytetracycline and oxolinic acid in the laboratory microcosms. Anoxic sediments were collected from a drug-free fish farm and mixed with either oxytetracycline or oxolinic acid (1.0 $\mu g \cdot g^{-1}$ of sediment). Half of the samples, four oxytetracycline and four oxolinic acid, were incubated at either 4°C or 17°C. HPLC analysis of samples collected from the microcosms periodically over 77 days showed that oxolinic acid and oxytetracycline declined rapidly within 20 days of mixing with the sediment. Oxolinic acid degraded more quickly than oxytetracycline at both temperatures. Degradation of both antibiotics was faster at 17°C than at 4°C. However, the authors appear to have conducted at least 22 independent t-tests on data without Bonferroni correction (Zar 1999). Thus, inferences on significant differences in the rate of decline between the two chemicals may be too liberal.

Capone et al. (1996) set-up microcosms in 40-L laboratory aquariums containing sediment collected at and 1 km from an Atlantic salmon farm from Puget Sound. Commercial salmon feed was added to each microcosm at a 20 $mg \cdot m^{-2}$ per day for 14 days (simulating sedimentation rate at a fish farm). Medicated feed was then added for 5 to 10 days followed by unmedicated feed for 60 days. Sediment samples were drawn from the mesocosms periodically for HPLC analysis of oxytetracycline and sulfamethoxine + ormetoprim. Generally, oxytetracycline residues were high at the start of the simulated post-treatment period and declined slowly over time. There appears to be a tremendous amount of variability among microcosms in oxytetracycline concentration, and no attempt was made to make statistical comparisons between sediment collected at the reference site (1 km for the farm) and sediment collected at the farm. As such, drawing inferences regarding the difference between farm sediment and non-farm sediment in oxytetracycline residue persistence is impossible.

Only six samples in the study by Capone et al. (1996) were analyzed for levels of sulfadimethoxine and ormetoprim. Of those, one had detectable levels of ormetoprim (0.2 $\mu g \cdot g^{-1}$) and sulfadimethoxine (0.4 $\mu g \cdot g^{-1}$).

All of the above studies examining drug residues in sediment have used HPLC to estimate drug concentrations. However, several authors (e.g. Smith et al. 1994a, b) have argued that HPLC overestimates the concentration of biologically active antibiotics, most notably oxytetracycline, because it is chelated by Ca^{2+} and Mg^{2+} ions present in water and sediments. The chelated form of oxytetracycline, although detectable by HPLC, is not a biologically active form and has no antimicrobial properties. Three microcosm studies examined the role of Ca^{2+} and Mg^{2+} ions on the activity of oxytetracycline. Lunestad and Goksoyr (1990) added 2.5% seawater, or either 10 mM Ca^{2+} or 10 mM Mg^{2+} ions to growth medium and attempted to culture several species of sediment bacteria. They found that the presence of seawater or Ca^{2+} or Mg^{2+} ions inhibited the effectiveness of oxytetracycline relative to growth media containing no seawater, Ca^{2+} or Mg^{2+}. Herwig et al. (1997) cultured several species of bacteria on growth medium containing either oxytetracycline or sulfamethoxine + ormetoprim and a range of seawater concentrations. They found that oxytetracycline was most effective at inhibiting bacterial growth at low salinity and its effectiveness gradually decreased as salinity increased. On the other hand, sulfamethoxine + ormetoprim was unaffected by salinity. The authors attributed the reduced effectiveness of oxytetracycline at higher salinity to the presence of Ca^{2+} and Mg^{2+} ions. On the other hand, Vaughn and Smith (1996) found that the presence of sediment, and not Ca^{2+} and Mg^{2+} ions, was responsible for reduction in oxytetracycline effectiveness.

In summary, several studies have examined the fate of antibiotics in the marine environment. However, information is lacking regarding the fate and effect of therapeutants in freshwater systems. In addition, knowledge gaps exist regarding the causal factors controlling the distribution, accumulation and persistence of chemicals.

ENVIRONMENTAL IMPACTS OF ANTIBIOTICS USED IN AQUACULTURE

Toxicity

Acute toxicity of oxytetracycline to fish is low (Bjorklund 1991). Fourteen days of exposure to 750 $mg \cdot kg^{-1}$ $fish \cdot day^{-1}$ did not result in mortality in rainbow trout, nor did a single dose at 10.5 $g \cdot kg^{-1}$ fish. Marking et al. (1988) estimated the LC_{50} of oxytetracycline for lake trout (*Salvelinus namaycush*) in 1 h of water-bath exposure to be 840 $mg \cdot L^{-1}$ and < 200 $mg \cdot L^{-1}$ if the duration of exposure was increased to 24 h or more. In addition, Marking et al. (1988) found that oxytetracycline present in feed had an effect on growth at doses that were four and five times recommended (1.1 g $oxytetracycline \cdot kg^{-1}$ feed), but the effect was small. Smaller doses had no effect on growth.

Some antibiotics have been shown to reduce fish immune responses. For example, oxytetracycline and florfenicol have been shown to inhibit lymphoid cell mitogenesis, and oxytetracycline reduces antibody production and white blood cells in rainbow trout (Lunden et al. 1998; Lunden and Bylund 2000). However, florfenicol does not appear to interfere with production of antibodies nor immune cells (Lunden et al. 1999). Sulfadiazine + trimethoprim had no effect on lymphoid cell mitogenesis (Lunden and Bylund 2000) and may in fact stimulate rainbow trout immune response (Lunden and Bylund 2002).

Seventeen per cent of 170 g Atlantic salmon treated with 155 to 200 mg oxytetracycline $kg \cdot day^{-1}$ had spinal deformities, often only visible on X-ray (Toften and Jobling 1996). Deformities did not appear until 44 days of continuous exposure. The dose and duration of dosing in the study are higher than normal, so the results are not relevant to farmed fish. However, the results may be significant to wild fish because they may have constant access to oxytetracycline containing feed under cage culture operations. Spinal deformities were not observed in 290 g Arctic charr at the same exposure level (Toften and Jobling 1996).

Wollenberger et al. (2000) examined acute and chronic toxicity of oxytetracycline and sulfadiazine (a component of sulfadiazine + trimethoprim) on *Daphnia magna*. No acute effect was observed for oxytetracycline or sulfadiazine at levels ranging between 7.8 to 230 $mg \cdot L^{-1}$ and 3.1 to 100 $mg \cdot L^{-1}$ for the two drugs respectively (Table 5). Chronic effects of oxytetracycline and sulfadiazine on reproduction were $EC_{50} = 46.2\ mg\cdot L^{-1}$ and 13.7 $mg\cdot L^{-1}$ respectively (EC_X, effective concentration, is the concentration that renders X% of the organisms immobile). Oxytetracycline (5.0 $\mu g\cdot L^{-1}$) had no influence on the hatch rate of rotifer eggs (Balompapueng et al. 1997). Holten Lützhøft et al. (1999) examined the impacts of oxytetracycline and the components of sulfadiazine + trimethoprim (sulfadiazine and trimethoprim) on freshwater primary producers. The EC_{50} values for oxytetracycline and sulfadiazine are lower for the freshwater cyanobacteria *Microcystis aeruginosa* than for the green algae *Selenasrtum capricornatum* and similar between the two species for trimethoprim (Table 5). The predicted no-effect concentration (PNEC) of oxytetracycline is 0.23 and 4.5 $\mu g\cdot L^{-1}$ for *M. aeruginosa* and *S. capricornatum* respectively (Jones et al. 2002 based on Holten Lützhøft et al. 1999).

Baguer et al. (2000) examined the toxicity of oxytetracycline to three soil invertebrates: *Folsomia fimitaria* (Collembola: Isotomidae), *Enchytraeus crypticus* (Enchytraeidae: Oligochaeta) and *Aporrectodea cliginosa* (Annelida: Oligochaeta). They found that the EC_{10} values (Table 5) for all three species are above those found in manure from terrestrial animal farms and are above those found in aquaculture farm sediments summarized previously in this review.

Experimental and statistical methods for determining NOECs and LOECs as well as their application have been criticized for their lack of relevance to real world situations. Typically, results from standardized laboratory toxicology studies have been used to extrapolate to ecological effects (Graney 1994). Baguer et al. (2000) and Hoekstra and van Ewijk (1993) both suggest changes to the experimental and statistical methodology used in toxicology studies. Baguer et al. (2000) suggest that mesocosm studies may shed light on ecosystem level processes. The uses of mesocosms in ecological impact assessment are reviewed in Graney et al. (1994).

Ecological Impact

Primary productivity influences the structure and function of ecological communities (Rosenzweig and Abramsky 1993 and Rosenzweig 1995 for reviews). Although nutrient loading is the most obvious potential impact of aquaculture on primary productivity, therapeutants may also have an impact. Antibiotics will affect non-target bacteria and, as mentioned above, can affect cyanobacteria and green algae. Bacteria are important for nutrient cycling and cyanobacteria and algae for primary production. Local communities could be affected if both trophic groups are eliminated from the area surrounding aquaculture operations.

Nitrosomonas spp. and *Nitrobacter* spp. are important bacteria for nutrient cycling in freshwater trophic webs converting ammonia (a toxic form of nitrogen) to nitrate (non-toxic form of nitrogen) (Ricklefs and Miller 2000). Klaver and Mathews (1994) examined the effect of oxytetracycline on *Nitromonas* and *Nitrobacter* in 91 laboratory microcosms. Initially, each microcosm contained 5 $mg\cdot L^{-1}$ NH_3-N. In addition, 0, 12.5, 25 and 50 $mg\cdot L^{-1}$ of oxytetracycline was added to each aquarium, and a known inhibitor of nitrification (533 $mg\cdot L^{-1}$ of N-Serve, Dow Chemical Co.) was added to a fifth microcosm as a positive control. This design was replicated four times for a total of 20 microcosms. Oxytetracycline greatly inhibited the activity of *Nitromonas* and *Nitrobacter* in the laboratory microcosms. For example, by Day 4, ammonia in the 0, 12.5, 25, 50 mg·L^{-1} oxytetracycline and N-Serve microcosms dropped to 1.9, 3.2, 3.8, 4.0 and 4.9 mg·L^{-1} respectively. At Day 9, ammonia was present at 0.9, 2.5, 3.7, 4.2 and 4.9 mg·L^{-1} in each of the microcosms respectively. These results show clearly that oxytetracycline inhibited the ammonia processing effect of *Nitromonas* and *Nitrobacter*.

Klaver and Mathews (1994) undertook their investigation because of a dilemma posed to catfish farmers. Rapid bloom and death of phytoplankton resulting from high fish feeding rates in catfish ponds leads to the presence of several forms of nitrogen, including high concentrations of ammonia and nitrites (Klaver and Mathews 1994). High concentrations of ammonia and nitrites often coincide with seasonal disease outbreaks in catfish ponds. Antibiotic therapy may help cure the disease, but at a cost of reduced conversion of toxic ammonia to non-toxic forms of nitrogen. Cage culture operations may be faced with the same ecological problem. As the studies of Bjorklund et al. (1990, 1991) and Samuelsen et al. (1992a) show, a great deal of organic matter can build up under cage culture facilities. Metabolism of this waste could cause build-up of ammonia in the sediments. Under normal conditions, *Nitrosomonas* and *Nitrobacter* would likely process the ammonia; but during disease outbreaks that call for drug use, ammonia may be able to build up.

Evolution of Resistance

The evolution of resistance is an inevitable consequence of antibiotic use (Stokes 2002), and this consequence is perhaps the most important implication of antibiotic use in aquaculture for a variety of reasons (Salyers et al. 2002). For example, resistant strains of bacteria require even higher levels of dosing to treat disease leading to more antibiotic reaching the natural environment (Bruun et al. 2003). Furthermore, the mechanism of drug resistance is plasmid encoded for many of the drugs in use, leading to the potential for transfer of resistance among species (Rhodes et al. 2000; Schmidt et al. 2001). The importance of the problem of resistance is evidenced in the large number of studies devoted to understanding antibiotic's role in the evolution and spread of drug resistant bacteria and in the initiation of antibiotic resistance monitoring programs by many governments (see Conly 2003). The general premise of many studies examining resistance is that as more antibiotic is introduced into the environment, the greater will be the incidence of antibiotic resistance in the bacterial flora (Smith et al. 1994a; Halling-Sorensen et al. 1998). Evidence supporting this premise has been shown in a number of studies (e.g. Schmidt et al. 2000; Bruun et al. 2003).

Resistance to antibiotics is present at low frequency in bacterial populations naturally (McPhearson et al. 1991; Johnson and Adams 1992; Spanggaard et al. 1993; de la Cruz et al. 2002; Walsh 2003). Antibiotic resistance comes at a cost to its bearer, and this cost keeps the number of resistant strains low relative to non-resistant strains (Palumbi 2001; Walsh 2003). However, when antibiotics are present, resistant strains have an advantage over non-resistant strains and predominate in the population. Antibiotic use allows the resistant strains a better chance to proliferate and allows the spread of resistance mechanisms through a variety of mechanisms (see de la Cruz 2002).

Studies of antibiotic resistance in aquaculture take two general forms: one in the natural world and one in laboratory microcosms. Both involve adding antibiotic and examining the subsequent number of resistant strains of bacteria present in the natural or artificial sediments. A brief summary of these studies is presented in Table 6.

Fish Farm Studies: All of the studies examining antibiotic resistance following drug therapy reviewed here demonstrated an increased frequency of resistance to several drugs across a variety of bacterial species. DePaola et al. (1995) observed rapid increase of resistance to oxytetracycline across a variety of bacterial species isolated from rearing pond water and fish intestines at a catfish farm in the United States. Roughly 0.3 and 8% of intestinal isolates from a control pond (received no medicated feed) were resistant to oxytetracycline in fall and spring respectively, and the percent of resistant isolates in treated pond (50 mg oxytetracycline$\cdot$kg^{-1} fish$\cdot$day^{-1} for 10 days) were as high as 48 and 82% during fall and spring respectively. Resistance levels in the untreated pond water was approximately 10%, whereas in the treated pond resistance rose as high as 90% during the spring. Resistance levels in the treated pond returned to background levels after 21 days post drug treatment.

McPhearson et al. (1991) examined bacterial resistance in two catfish ponds (one that had received therapy recently and one that had not) and in a nearby river (control). They found that the incidence of resistance to oxytetracycline across all bacterial species examined was 0.8% in the river, 25.0% in the untreated pond and 69.1% in the treated pond. Both *A. hydrophilus* and *E. coli* from the river were not resistant to oxytetracycline. However, 22 and 62% of *A. hydrophilus* and 63 and 100% of *E. coli* isolates were oxytetracycline resistant in the untreated and treated ponds, respectively.

In a similar study, Spanggaard et al. (1993) found that oxytetracycline and oxolinic acid resistance in an unpolluted Danish stream were 6% and 16% respectively. Resistance frequency for both drugs was much higher at three rainbow trout farms (15% and 27% for oxytetracycline and oxolinic acid respectively). The authors reported that the level of resistance in the fish farm environments was not significantly different from the unpolluted stream environment. However, Spanggaard et al. (1993) conducted χ^2 contingency table analysis on a large table of % of strains growing at different drug concentrations (10 drug concentrations X 3 sample collection sources X 2 habitats X 2 drugs). χ^2 may not be the appropriate analysis for this study.

Rangdale et al. (1997) surveyed 48 isolates of *Flavobacterium psychrophilum* for resistance to a variety of antibiotics. They found that sulfadiazine + trimethoprim and oxytetracycline each had a negligible effect on all strains (i.e. the strains are resistant), whereas florfenicol was effective at inhibiting all strains (i.e. resistance was minimal). Similarly, Bruun et al. (2000) collected bacteria from a Danish rainbow trout farm that had used oxytetracycline, sulfadiazine + trimethoprim and florfenicol. They found strains of *F. psychrophilum* that were resistant to oxytetracycline. All strains were resistant to sulfadiazine + trimethoprim, but none was resistant to florfenicol. They suggested that this bacterium must have intrinsic resistance to sulfadiazine + trimethoprim because even the standard was affected little by the drug. The results of this study are similar to those of others with regard to oxytetracycline, sulfadiazine + trimethoprim and florfenicol. For example, Kim and Aoki (1993) and Ho et al. (2000) found many strains across a variety of bacterial isolates from cultured fish in Asia that were resistant to oxytetracycline and sulfadiazine + trimethoprim, but few of the isolates were resistant to florfenicol. On the other hand, whereas Miranda and Zemelman (2002a, b) have found elevated oxytetracycline resistance at freshwater Atlantic salmon farms in Chile, they have also found resistance to florfenicol ranging from 20 to 80% of isolated strains sampled across four farms (three land-based and one lake-cage). A possible reason for the higher rates of florfenicol resistance in Chile is a longer history of florfenicol use relative to Asian and Scandinavian aquaculture.

Guardabassi et al. (2000) examined resistance in *Acinetobacter* spp. (as an indicator of overall bacterial resistance) at a single freshwater rainbow trout farm in Denmark undergoing treatment with oxolinic acid. Samples were collected at the farm inlet, a pond, the farm outlet and a location 300 m downstream from the farm. Mean resistance levels for oxolinic acid among all bacterial isolates identified were 0% at the inlet, 46% in the pond, 21.9% at the effluent and 52.4% 300 m downstream. Surprisingly, the isolates also exhibited resistance to oxytetracycline at each of the locations (0.9%, 4.0%, 10.5% and 0% for the inlet, pond, outlet and downstream locations respectively).

Schmidt et al. (2000) examined spatial changes in resistance among strains of *Aeromonas salmonicida*, *Yersinia ruckeri* and *F. psychrophilum* along a stream subject to inputs from four land-based rainbow trout farms. If the effects of antibiotic use on farms were far reaching, the most downstream farm was expected to have the highest additive inputs of antibiotics and consequently the highest levels of resistance. However, the results suggest that the impacts of antibiotics on resistance are local because the stream locations with the highest levels of resistance were associated with the farms using the most antibiotics. The most downstream farm used the least amount of antibiotic and had the lowest associated levels of resistance.

Several studies have examined resistance under marine fish farms. For example, Bjorklund et al. (1991) found that bacterial isolates from sediments under a farm using oxytetracycline and oxolinic acid had low sensitivity to oxytetracycline but not oxolinic acid. Unfortunately, background resistance at non-farm sites was not determined, so sensitivity of the bacterial isolates in their study may or may not be abnormally high.

Samuelsen et al. (1992a) examined oxytetracycline resistance under three salmon cages along the coast of Norway. Two of the three cages underwent oxytetracycline therapy (75 $mg \cdot kg^{-1}$ per day for 10 days), and the third was untreated. Resistance levels were high under all three cages immediately following therapy and decreased rapidly over 50 days. Again, no background or initial rates of resistance were determined. The authors could not isolate the effects of oxytetracycline on resistance from the effects of decomposing feed under the cage. Kerry et al. (1996) also found that oxytetracycline resistance fell rapidly following cessation of oxytetracycline therapy at an Irish marine farm. Background resistance (at a non-farm site) was 1.2%. Resistance at the farm was 16% on the day drug therapy ended, 10% and 3% at Days 10 and 32, and had decreased to background levels by Day 73 post-therapy.

Herwig et al. (1997) examined oxytetracycline and sulfamethoxine + ormetoprim resistance in bacteria from sediments under three Atlantic salmon farms and several non-farm locations in Puget Sound. The three farms differed in the total amount of antibiotics used. Resistance to both oxytetracycline and sulfamethoxine + ormetoprim appear to be roughly double under the farms compared to non-farm site. In addition, resistance was most frequent at the farm receiving the greatest amount of drug therapy.

Two other studies examined the frequency of resistant bacteria in wild organisms living near fish farms. Bjorklund et al. (1990) found oxytetracycline resistant strains of *Vibrio anguillarum* and *A. salmonicida* in the guts of roach and bleak. Although no comparison was made in this study with bacterial flora of roach and bleak caught elsewhere, the resistance levels reported by Bjorklund et al. (e.g. $MIC_{oxytetracycline} = 18.8\ \mu g \cdot g^{-1}$ for *Vibrio* spp.) were much higher than reported previously (e.g. $MIC_{oxytetracycline} = 1.56\ \mu g \cdot g^{-1}$ for *V. anguillarum*). Ervik et al. (1994) found that 0.6% of bacteria associated with blue mussels near a fish farm were resistant to oxytetracycline prior to drug therapy. However, 20% were resistant following drug therapy.

Nygaard et al. (1992) placed 18 boxes (60 X 40 X 10 cm deep) of sediment on the bottom (20 m depth) near a Norwegian Atlantic salmon farm. Each box of sediment contained either no antibiotic (n=6), 50 ppm oxytetracycline (n=6) or 10 ppm oxolinic acid (n=6). Resistance of microorganisms in each box was determined three times over the course of a year. Background resistance in the sediments at the start of the experiment (0 months) was 5% and 7% for oxytetracycline and oxolinic acid respectively. At 10 months, resistance had increased to 10 and 14% and at 12 months to 16% and 20% for oxytetracycline and oxolinic acid respectively.

In summary, the field studies suggest a relationship between increased drug use and increased resistance. However, knowledge is incomplete regarding the role of organic load, water chemistry and sediment adsorption properties in affecting bacterial resistance.

Microcosm Studies: Kerry et al. (1996a) simulated under-cage sediments in 50-mL vials to determine the effect of oxytetracycline on bacterial metabolism. Each vial contained 14 g sediment, 20 mL water and 0.8 g fish feed (control vials had no feed). Oxytetracycline was added at various concentrations ($0.1\text{-}600\ \mu g \cdot g^{-1}$). Initial metabolic rate in the vials was highest with low oxytetracycline concentration and lowest in the high oxytetracycline concentration. However, after about 50 h, metabolic rate in the high oxytetracycline vials caught up with that in the low oxytetracycline vials. This observation suggests that resistant strains increased in frequency in the high oxytetracycline vials, a prediction that was confirmed by subsequent culturing of the bacteria from the various oxytetracycline treatments on oxytetracycline containing growth medium.

Herwig and Gray (1997) set up microcosms in 38-L aquariums, each with 15.5 cm of sediment and 40 g·m^2·day of fish feed. Oxytetracycline or sulfadiazine + trimethoprim were added to treatment aquaria (incorporated in the feed as 0.83% oxytetracycline or 0.5% sulfadiazine + trimethoprim of feed mass), and control aquaria received only feed. Addition of sulfadiazine + trimethoprim increased the level of resistance to sulfadiazine + trimethoprim relative to the controls. Addition of oxytetracycline increased the level of resistance to both oxytetracycline and sulfadiazine + trimethoprim relative to the control.

In a study similar to that by Kerry et al. (1996a), O'Reilly and Smith (2001) found that the minimum effective concentration of oxytetracycline in 100-mL microcosms to produce resistant strains of bacteria was 20 mg·L^{-1}. Concentrations below 20 mg·L^{-1} were not successful in promoting high frequencies of resistance.

The above studies have demonstrated evidence for a role of antibiotics in promoting increased resistance. However, studies by Kapetanaki et al. (1995) and Vaughan et al. (1996) suggest that factors other than antibiotics, which are confounded in the studies above, can also lead to increased rates of resistance. For example, Kapetanaki et al. (1995) observed increased rates of oxytetracycline resistance among bacteria with increasing amounts of sterile fish feed, but in the absence of any antibiotic in 39 X 30 X 27 cm microcosms. Additionally, Vaughan et al. (1996) added 6 cm of river sediment to each of two 29 X 24 X 23 cm microcosms. In addition, 16 cm of sterile commercial fish feed was added to one of the microcosms. The researchers observed a 10-fold increase in the rate of resistance in the feed microcosm relative to the no feed microcosm. The authors concluded that oxytetracycline resistance can occur in the presence of uneaten feed and that field studies documenting increased oxytetracycline resistance under fish farms may incorrectly attribute this increase to oxytetracycline treatment at the fish farm. Either scenario is feasible because the competitive advantage of non-resistant forms could be lost due to antibiotic presence of by non-limited food resources. Unfortunately, Vaughan et al. (1996) did not include an oxytetracycline treatment in their microcosm research, so there is now way to know the relative effects of oxytetracycline and high levels of organic matter on the evolution of oxytetracycline resistance. The laboratory experiments by Kapetanaki et al. (1995) and Vaughan et al. (1996) demonstrate that increased levels of bacterial drug resistance can arise independent of the presence of a drug.

FUNGICIDES AND DISINFECTANTS

Fungicides and disinfectants have been included in the same section because their method of use and the chemicals involved are the same in some cases. For example, treatment of external fungal or bacterial infections most often involves immersion of fish in a static bath containing the therapeutic agent. There are three common chemicals used to treat fungal infections (hydrogen peroxide, formalin and sodium chloride) and four used as disinfectants (hydrogen peroxide, N-chloro-paratoluenesulfonide trihydrate, sodium chloride and iodophore compounds) in Canadian freshwater aquaculture (Table 1). Fungicides are used most commonly to treat (or prevent) external infections of fungi belonging to Saproligiaceae which cause fungal disease of embryos through adults (Noga 1996). Disinfectants are used to treat (or prevent) external bacterial infections such as bacterial gill disease, caused by *Flavobacterium branchiophila* (Noga 1996). Treatment of infected embryos, juveniles or adults typically takes place in a static immersion bath. This form of treatment is most often applied to embryos and juveniles inside a hatchery, but has also been applied to adult fish in culture ponds (e.g. Chiayvareesajja and Boyd 1993; Boyd and Massaut 1999). Treatment concentrations for hydrogen peroxide (30% active ingredient), formalin (37% formaldehyde solution), sodium chloride and N-chloro-paratoluenesulfonide trihydrate in static baths vary depending on fish species and density, ranging from 50 to 250 $\mu L \cdot L^{-1}$, 500 to 2000 $\mu L \cdot L^{-1}$, 100 to 30000 $mg \cdot L^{-1}$, and 10 $mg \cdot L^{-1}$ respectively (Noga 1996; Sanchez et al. 1996; Schreier et al. 1996; Boyd and Massaut 1999; Gaikowski et al. 1999). Formalin and hydrogen peroxide concentrations used to treat the aquaculture ponds were 10 $mg \cdot L^{-1}$ and 100 $kg \cdot ha^{-1}$ respectively (Chiayvareesajja and Boyd 1993; Boyd and Massaut 1999).

Very little has been published between 1990 and 2001 that has bearing on the environmental fate and effect of fungicides and disinfectants (no published studies directly examined this issue). However, several studies have examined tissue deposition, toxicity and stress responses for these chemicals in fish to determine appropriate use rates in aquaculture practice. For example, two studies examined the fate of formalin in cultured fish (Xu and Rodgers 1993; Jung et al. 2001). Both of these studies found that tissue levels of formalin increased significantly relative to a control only at high concentrations (> 300 $mg \cdot L^{-1}$) (Jung et al. 2001) and returned to normal/control levels within one to five days following immersion in formalin baths (Xu and Rodgers 1993; Jung et al. 2001).

Several studies have investigated toxicity of formalin and hydrogen peroxide in treatment of fungal infections (see Table 3 for a summary of toxicity values). Toxicity of the various chemicals used as fungicides varies among fish species and among life stages within a species (Howe et al. 1995; Rach et al. 1997a, b, 1998; Gaikowski et al. 1998, 1999). Schreier et al. (1996) showed that healthy rainbow trout embryoes eclose at the same rate (roughly 88%) for formalin (1500 $\mu L \cdot L^{-1}$), peroxide (1500 $\mu L \cdot L^{-1}$) and salt (30 $g \cdot L^{-1}$), which was the same as the hatch rate for the control. Carp have high survival in high concentrations of either peroxide (Rach et al. 1998) or formalin (Rach et al. 1997b). On the other hand, sturgeon have low survival in high formalin exposure (Rach et al. 1997b) and high survival in high peroxide exposure (Rach et al. 1998). In general, cool- and warm-water species are more sensitive to hydrogen peroxide than are cold-water species (Gaikowski et al. 1999). Rainbow trout embryoes are much less susceptible to hydrogen peroxide and formalin than are older stages (Howe et al. 1995; Rach et al. 1997a; Gaikowski et al. 1998). Also, Gaikowski et al. (1998) have shown that toxicity of hydrogen peroxide varies among strains of rainbow trout. For example, survival of rainbow trout embryoes from Washington State is 67% in 3000 μL·L^{-1} hydrogen peroxide. However, survival of Ganaraska and Skamania strains of this species is only 28 and 3.5% respectively.

N-chloro-paratoluenesulfonide trihydrate immersion (10 mg·L^{-1} 1 h per week for four weeks) has no immediate effect on O_2 consumption (Speare et al. 1996) or plasma cortisol levels (Sanchez et al. 1997) in rainbow trout relative to sham treated fish. This suggests that N-chloro-paratoluenesulfonide trihydrate does not additionally stress rainbow trout beyond that provided by handling. Secondary stress measures (glucose, haematocrit, sodium chloride) also show no influence of N-chloro-paratoluenesulfonide trihydrate immersion over longer periods (11 weeks) of weekly exposure. However, biweekly 1-h exposure results in reduced growth resulting most likely from decreased feed conversion efficiency (no loss of appetite was observed) (Sanchez et al. 1996).

Many of these chemicals have low toxicity at therapeutic concentrations and are further diluted before being released to the environment, suggesting a low likelihood of acutely toxic effects. For example, the freshwater, land-based aquaculture facility in Smith et al. (1994a) and Vaughan et al. (1996) discharges approximately 46,080,000 L of water per day. The net discharge of hydrogen peroxide following static bath therapy at 200 μL·L^{-1} in a 2000-L rearing tank would be 400,000 μL. The concentration of released hydrogen peroxide for the day would be 2.17 μL·L^{-1}, well below any values reported in Table 3. One point to keep in mind is that the toxicity data reviewed here and summarized in Table 3 are for acute exposure and not for chronic exposure. Concentrations having a toxic effect following chronic exposure may be lower than those for acute exposure. However, information is generally lacking regarding the chronic toxicity and effects of chemotherapeutants to fish and other freshwater organisms.

ANAESTHETICS

One anaesthetic is used in Canadian aquaculture (Table 1). Anaesthetics are used to subdue fish and subsequently reduce stress responses caused by handling. No studies were found in the recent literature regarding the environmental fate and effect of anaesthetics aside from the study by Keene et al. (1998), in which the LC_{50} was determined for clove oil (Table 3).

PIGMENTS

Carotenoid pigments are responsible for the red colouration of salmonid flesh and are added to aquaculture feed (most commonly used are astaxanthin and canthaxanthin) to enhance flesh colour in cultured salmonids (Guillou et al. 1995; Metusalach et al. 1997). No studies have been published on the environmental fate and effect of carotenoid pigments introduced in fish feed. Caretonoid pigments could accumulate in sediments as they are non-water soluble molecules and stable in the absence of light. However, Jorgensen and Skibsted (1993) have shown antioxidant behaviour in caretonoids to be most pronounced at low oxygen partial pressure.

HORMONES

Salmonid aquaculture production may be enhanced by culturing females only, a condition that requires sex inversion (manipulation of sex phenotype). Sex inversion involves exposing juvenile fish to 17-alph-methyltestosterone. Exposing females to this hormone causes them to develop phenotypically as males while retaining their female genotype. The sex inverted fish are crossed with non-sex inverted females when they become sexually mature. Whereas the phenotype cross is male X female, the genotype cross is female X female and produces a brood of offspring that are all genotypically and phenotypically female. Two methods can be used to expose juvenile fish to 17-alph-methyltestosterone: immersion or incorporation in feed (Johnstone and MacLauchlan 1994). Both methods have the potential to release 17-alph-methyltestosterone into the environment, especially when exposure takes place via incorporation with feed (an inefficient method). There is considerable concern over release of this hormone into the environment (Johnstone and MacLachlan 1994), but I have found no studies examining its environmental fate or effect.

KNOWLEDGE GAPS

Research on the fate and effect of therapeutants in freshwater systems.

The vast majority of published research on the fate and effect of aquaculture chemotherapeutants is based on marine sea-cage models. Although some freshwater cage-culture operations exist in Canada, the majority of the freshwater aquaculture is land-based hatchery, pond or recirculating systems, so much of the research has no direct bearing on Canadian freshwater aquaculture. In addition, although the general practices of medicating fish are similar between freshwater and marine cage operations, the differences in the physical and chemical characteristics of the water and bottom substrates make the inferences regarding antibiotic fate in freshwater doubtful.

Research is needed to identify the causal factors controlling the distribution, accumulation and persistence of chemicals.

Several studies have shown some clear examples of the fate and impact of antibiotics in the environment. The gastrointestinal tract in fish absorbs only a small amount of the antibiotic in feed. The remainder is released to the environment. Wild fish will eat medicated feed that passes out of a cage. Antibiotic residues build up under cages and gradually dissipate over varying amounts of time.

Feeding and chemical treatment methods on the fish farm are potentially the largest contributor to accumulation of antibiotics in the wild. The research reviewed here points to several mechanisms that need to be further investigated to understand various factors that additionally influence antibiotic accumulation and persistence. For example, studies have shown that the method of incorporating an antibiotic with feed influences how fast the antibiotic is lost to the surroundings (Duis et al. 1994; Rigos et al. 1999). Recent advances in technology allow aquaculturists to minimize the amount of feed (and therefore antibiotics during therapy) reaching the sediment (e.g. Juell et al. 1993; Ervik et al. 1994; Foster et al. 1995; Madrid et al. 1997). Therefore, the next logical issue regarding minimizing the amount of antibiotic reaching the environment is to find methods of incorporation that reduce direct loss of antibiotic from feed to the surrounding water.

In addition, several studies have suggested that currents play an important role not just in the initial dispersal of antibiotics around a farm, but in their rate of disappearance from the sediments as well (Bjorklund et al. 1990, 1991; Samuelsen et al. 1992a; Coyne et al. 1994). No field studies have been conducted to test this hypothesis explicitly. In addition, currents probably influence the amount of organic sludge that builds up under a cage facility. Samuelsen et al. (1992a), Coyne et al. (1994) and Hektoen et al. (1995) demonstrated that drug residues persist longer at greater sediment depths, and Kapetanaki et al. (1995) and Vaughan et al. (1996) have shown that resistance can arise when high organic load is present in the absence of antibiotics. So research that attempts to address the impact of current also needs to take into account the potential confounding effects of organic sludge build-up.

Research is needed regarding the factors affecting bacterial resistance to antibiotics. Various studies have also shown that the direct relationship between drug use and resistance is not all that clear. Studies vary with regard to the predicted relationship between increased drug use and increased resistance. Although field studies unanimously supported the prediction, they did not control for organic load, which has been shown to be a potentially confounding variable in laboratory microcosm studies (Kapetanaki et al. 1995; Vaughan et al. 1996). Water chemistry and sediment adsorption properties and their interaction with drugs are other important factors identified for further investigation (Lunestad and Goksoyr 1990; Vaughan and Smith 1996; Herwig et al. 1997). Calcium and magnesium ion concentrations were shown to have an influence on bioactivity of oxytetracycline (Lunestad and Goksoyr 1990). However, Vaughan and Smith (1996) showed that sediment alone could reduce the bioactivity of oxytetracycline. Clearly this needs further attention because the main issue driving the research on Ca^{2+} and Mg^{2+} in the first place was their high concentrations in seawater. Concentrations of these ions are much lower in freshwater. If they are a significant factor in reducing oxytetracycline effectiveness, then the availability of bioactive oxytetracycline is potentially much greater in freshwater than in seawater.

Research is needed to examine the chronic toxicity of antibiotics and other chemotherapeutants to fish and other freshwater organisms. Although there have been many studies published that examine the fate and effect of antibiotics on the environment, none of them directly address the remaining chemotherapeutants. Acute toxicity of therapeutic concentrations of fungicides, disinfectants and anesthetics is low to several Canadian freshwater species and further dilution in effluent suggests that fungicides and disinfectants are unlikely to cause acute toxicity to fish in the environment. However, examination of the effects of chronic exposure to low concentrations to fish and other organisms remains to be addressed.

There is a need to develop standard sampling design and analytical protocols in aquaculture science.

Controls: Several studies examining drug residues under fish farms did not examine drug residues at control and non-farm sites. Without such controls in place, one could always argue that changes in drug residues observed under a fish farm facility reflect natural changes. Demonstrating that sediment drug residues are low and unchanging at a control site, while those under a fish farm receiving drug therapy rose sharply, is much stronger evidence of an effect of aquaculture than monitoring under a single farm without a control.

Replication: Many published surveys of drug residue persistence and resistance around farms were either not replicated or replicated insufficiently. Collecting several samples at a single farm or from a single microcosm is pseudoreplication. Appropriate replication requires sampling replicate farms or replicate microcosms (Hurlburt 1984). This matter is not trivial because sampling the same item repeatedly leads to an underestimate of the error variance and subsequently to artificially inflated t and F statistics. In addition, sampling a single farm makes it very hard to make broad inferences regarding the general impacts of aquaculture practice, including antibiotic use. Intensive sampling at a single site provides information about that site only. Activities of several investigators working independently could provide replication.

Protocols: There is little standardization of protocols among laboratories for nearly all techniques. For example, appropriate methods for determining drug residue concentration in sediments are not standardized. HPLC seems to be the analytical method of choice, probably because it is less labour intensive and faster than using bioassays to determine the active levels of drugs in sediment samples as done by O'Reilly and Smith (2001). However, some authors argue that HPLC overestimates the biologically active form of oxytetracycline and other drugs because they are chelated by Ca^{2+} and Mg^{2+} ions or adsorbed to sediment particles (Smith et al. 1994b). Research is needed to determine the relationship between HPLC drug residue results and the bioactivity (bioassay) of that drug under different environmental conditions.

Methods for determining drug resistance vary among laboratories (Table 7). Some labs plate a bacterial strain on two growth media that are identical except that one contains an antibiotic that is of interest. Resistance is calculated as a percent of colonies growing on the antibiotic-containing medium relative to the antibiotic-free medium (# colonies on antibiotic medium / # colonies on no-antibiotic medium * 100). Higher percentages mean higher levels of resistance. Others examine resistance by estimating the minimum inhibitory concentration (MIC), which is the lowest concentration of antibiotic that inhibits colony growth in a strain of bacteria. Bacteria strains with higher MICs are assumed to be more resistant to the antibiotic. Other labs use the zone of inhibition to determine drug resistance. The zone of inhibition is the size of the area around an antibiotic disc placed on a culture of bacteria. The smaller the zone, the greater is the resistance. It is difficult to make direct comparisons of resistance among labs that use different techniques. Further complicating the issue is the use of different culture media used for determining resistance among laboratories (see Table 7 for a summary of the diverse media used in the studies reviewed here). Science needs a standard set of accepted protocols to determine if results are real or artifacts of different experimental or analytical approaches. A set of standardized methods following those outlined in Alderman and Smith (2001) and NCCLS should be developed.

There is a need for an inventory of usage patterns that includes reports of what is used, where and in what amount.

Documenting the fate and impact of chemotherapeutants under various conditions in freshwater systems is a step in assessing the risk posed by these chemicals. However, an equally critical element in establishing such a risk is knowledge of how much of a chemical is used and where and when it is used relative to sensitive ecological parameters and human activities (Health Canada 2002). Presently in Canada, there is no standardized reporting of antibiotic use in aquaculture (Health Canada 2002). At present, only data on annual production of medicated feed are available nationally (Health Canada 2001b), and total usage is available only for the province of British Columbia[3]. Prescription and over the counter use are not available. These two components of use, 'over the counter' use in particular, must be known to adequately address risks associated with development of antimicrobial resistance (Health Canada 2002).

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Table 1. List of chemotherapeutants used in Canadian freshwater aquaculture.

Application	Common Name	Commercial Name	Approved by Health Canada for Use in Aquaculture[a]
Antibiotic	Florfenicol	Aquaflor/Nuflor	Yes
	Oxytetracycline	Terramycin aqua	Yes
	Sulfadiazine + trimethoprim	Tribrissen 40	Yes
	Sulfadimethoxine + ormetoprim	Romet 30	Yes
Fungicides	Formalin	Parasite S	Yes
	Hydrogen peroxide	Perox-Aid	Yes
Disinfectants	Hydrogen Peroxide	Perox-Aid	Yes
	N-chloro-paratoluenesulfonide trihydrate	Chloramine-T	No
	Sodium chloride		No
	Sodium hypochlorite		No
	Iodophore copounds	Wescodyne	No
Anaesthetic	Tricaine methansulfonate	MS-222	Yes
	Eugenol (Clove Oil)	Aqui-S	No
Hormones	17-α-methyl testosterone	Methyltestosterone	No
Food additives	Pigments	Carophyl Pink	Yes

[a] Health Canada, Food Safety Assessment Program, June 2001:
http://www.hc-sc.gc.ca/vetdrugs-medsvet/aquaculture_e.html [accessed 08/05/2003]

Table 2. Physical properties of chemotherapeutants used in Canadian freshwater aquaculture. Data presented are from Budavari et al. (1996) unless noted otherwise.

Chemical	Molecular Formula	Molecular Weight	Solubility in Water	LD_{50} in Mice	Maximum Residue Limit (ppm) [a]	Tissue Elimination Half-life (h)
Florfenicol	$C_{12}H_{14}Cl_2FNO_4S$	358.22	soluble	-	0.8	12.2[c]
Oxytetracycline-HCl	$C_{22}H_{24}N_2O_9$	460.44	1 g/ml[b]	-	0.1	60.3[d]
Sulfadiazine	$C_{10}H_{10}N_4O_2S$	250.28	13 mg/100 ml (pH 5.5) 200 mg/100 ml (pH 7.5)	-	0.1	3-7[e]
Trimethoprim	$C_{14}H_{18}N_4O_3$	290.32	0.04 g/100 ml	7 g/kg	0.1	2-3[e]
Sulfadimethoxine	$C_{12}H_{14}N_4O_4S$	310.33	4.6 g/100 ml (pH 4.1) 58.0 g/100 ml (pH 7.06) 5170 g/100ml (pH 8.7)	> 10 g/kg	0.1	9.9[f]
Ormetoprim	$C_{13}H_{16}N_4O_2$	260	-	-	0.5[g]	25.6[f]
Formaldehyde	CH_2O	30.03	miscible	0.8 g/kg	-	-
N-chloro-paratoluenesulfonide trihydrate	$C_7H_7ClNNaO_2S$	227.65	soluble		-	-
Eugenol	$C_{10}H_{12}O_2$	164.20	insoluble	3 g/kg	-	-
MS-222	$C_{10}H_{15}NO_5S$	261.30	1.0 g/0.8 ml		not available	-

[a] Maximum Residue Limit refers to the maximum concentration of residue allowed by Health Canada in edible tissue (Health Canada, Food Safety Assessment Program, June 2001: http://www.hc-sc.gc.ca/food-aliment/english/organization/food_safety_assessement/aquacult/aq-e00.htm [accessed 9 August 2001])

[b] Although oxytetracycline hydrochloride is water soluble, oxytetracycline is only slightly soluble in wa ter.

[c] Atlantic salmon; USP (2001a): http://www.usp.org/veterinary/monographs/florfenicol.pdf [accessed 9 August 2001]

[d] Rainbow Trout; Bjorklund and Bylund (1991)

[e] Horse; USP (2001b): http://www.usp.org/veterinary/monographs/potentiated_sulfonamides.pdf [accessed 9 August 2001]

[f] Atlantic salmon; Treves-Brown (2000)

[g] Salmonids - Canadian Food Inspection Agency, Approved Therapeutants for Aquaculture Use, Bulletin #9: http://www.inspection.gc.ca/english/anima/fispoi/manman/samnem/bull9e.shtml [accessed 24 March 2003]

Table 3. List of indices searched for this review.

- Biological and Agricultural Index
- Biological Abstracts
- Biology Digest
- Environmental Sciences and Pollution Management, which includes:
    - Agriculture and Biotechnology Abstracts
        - Aquatic Pollution and Environmental Quality
    - Bacteriology Abstracts
    - Ecology Abstracts
    - Digests of Environmental Impact Statements
    - Health and Safety Science Abstracts
    - Pollution Abstracts
    - Risk Abstracts
    - Toxicology Abstracts
    - Water Resource Abstracts
- Chemical Abstracts
- Medline
- Toxline

Table 4. Accumulation and persistence of chemotherapeutants in sediments near aquaculture facilities.

Study	Farm Type	Drug(s) Used[a]	Amount Used	Monitoring Duration (days)	Depth in Sediment (cm)	Concentration (μg/g)		Half-life (days)
						Initial	End of Study	
Bjorklund et al. 1990	Marine Cage	OTC	1.6 kg	8	0-5	0.18	< 0.01	-
			1.78 kg	308	0-5	2.0	1-4.4	-
Bjorklund et al. 1991	Marine Cage	OTC	2.6 kg	12	-	2.2	2.5	-
			5.3 kg	12	-	4.2	3.6	-
			0 kg	12	-	-	-	-
			0 kg	12	-	-	-	-
			2.6 kg	12	-	0.6	0.8	-
Samuelsen et al. 1992a	Marine Cage	OTC	0 kg	560	0-12	25	≈ 1	125
			1.7 kg	560	0-12	290	≈ 10	142
			0.863 kg	560	0-12	190	0	89
Capone et al. 1996	Marine Cage	OTC	186	120	0-2	1.5	0.2	-
			9.5	120	0-2	0.9	0	-
			0.3	120	0-2	0.5	-	-
Coyne et al. 1994	Marine Cage	OTC	47.05	66	0-2	9.9	1.6	16
			28.25	71	0-2	10.9	< 0.01	13
Kerry et al. 1996	Marine Cage	OTC	20 kg	5	0-2	-	1.3-4.5	-
Bebak-Williams et al. 2002	Re-circulating Tank	OTC	65.88 g	40	N/A	≈ 0.5 μg/l[b]	0	-
						0.13 μg/g[c]	0	-
			58.56 g	31	N/A	≈ 0.5 μg/l[b]	0	-
						0.15 μg/g[c]	0	-
Hektoen et al. 1995	Field Microcosm	OTC	200	230	0-2/5-7	200/200[d]	40/160[d]	151/>300[d]
		SDZ	5	230	0-2/5-7	5/5[d]	0.1/1.3[d]	50/100[d]
		TRM	1	230	0-2/5-7	1/1[d]	0.1/0.35[d]	75/100[d]
		FLO	50	230	0-2/5-7	50/50[d]	0/0[d]	1.7/7.3[d]

* - no data reported; [a] OTC – oxytetracycline; SDZ – sulfadiazine; TRM – trimethoprim; FLO - florfenicol; [b] concentration in tank water; [c] concentration in biofilter sand; [d] refers to 0-1 cm / 5-7 cm depth in sediment

Table 5. Summary of toxicity values for chemotherapeutants included in the review.

Chemical[a]	Species	Exposure			Parameter	Study[c]
		Conc.	Time	Temp. (°C)		
OTC	*D. magna*	1.0-100 mg/l	48 hrs	-	EC_{50} = 1000.0 mg/l	1
	Folsimia fimetaria	0-5000 mg/kg	21 days	20	$NOEC_S$ => 5000 mg/kg soil	2
					$NOEC_R$ => 5000 mg/kg soil	2
					$LOEC_S$ > 5000 mg/kg soil	2
					$LOEC_R$ > 5000 mg/kg soil	2
					$LC/EC_{10\text{-}S}$ > 5000 mg/kg soil	2
					$LC/EC_{10\text{-}R}$ > 5000 mg/kg soil	2
					$LC/EC_{50\text{-}S}$ > 5000 mg/kg soil	2
					$LC/EC_{50\text{-}R}$ > 5000 mg/kg soil	2
	Enchytraeus crypticus	0-5000 mg/kg	21 days	20	$NOEC_S$ = 3000 mg/kg soil	2
					$NOEC_R$ = 2000 mg/kg soil	2
					$LOEC_S$ = 5000 mg/kg soil	2
					$LOEC_R$ = 3000 mg/kg soil	2
					$LC/EC_{10\text{-}S}$ = 4201 mg/kg soil	2
					$LC/EC_{10\text{-}R}$ =134 mg/kg soil	2
					$LC/EC_{50\text{-}S}$ > 5000 mg/kg soil	2
					$LC/EC_{50\text{-}R}$ > 2701 mg/kg soil	2
	Aporrectodea caliginosa	0-5000 mg/kg	21 days	15	$NOEC_S$ = 3000 mg/kg soil	2
					$NOEC_R$ => 5000 mg/kg soil	2
					$LOEC_S$ = 5000 mg/kg soil	2
					$LOEC_R$ > 5000 mg/kg soil	2
					$LC/EC_{10\text{-}S}$ = 3251 mg/kg soil	2
					$LC/EC_{10\text{-}R}$ = 2127 mg/kg soil	2
					$LC/EC_{50\text{-}S}$ > 5000 mg/kg soil	2
					$LC/EC_{50\text{-}R}$ > 5000 mg/kg soil	2

Table 5. Continued.

Chemical[a]	Species	Exposure Conc.	 Time	 Temp. (°C)	Parameter	Study[c]
OTC (cont'd)	*Microcystis aeruginosa*	0.04-0.36 mg/l	7 days	21	EC_{50} = 0.207	3
	Rhodomonas salina	0.8-3.0 mg/l		21	EC_{50} = 1.6	3
	Selenastrum capricornutum	3.0-12.0 mg/l		23	EC_{50} = 4.5	3
	Rotifers	5 µg/l	60 min	25	% Hatch = 28.0	4
SDZ	*D. magna*	9.4-150 mg/l	24		LOEC = 150	1
	D. magna	9.4-150 mg/l	48		EC_{50} = 221	1
	Microcystis aeruginosa	0.02- 0.18 mg/l	7 days	21	EC_{50} = 0.135	3
	Rhodomonas salina	5.0-45 mg/l		21	EC_{50} = Out of Measurable Range	3
	Selenastrum capricornutum	3.0-27 mg/l		23	EC_{50} = 7.8	3
TMP	*Microcystis aeruginosa*	100-132 mg/l	7 days	21	EC_{50} = 112	3
	Rhodomonas salina	5.0-20 mg/l		21	EC_{50} = 16	3
	Selenastrum capricornutum	30-270 mg/l		23	EC_{50} = 130	3
Formalin	*Stizostedion vitreum*	1500 µl/l	45 min	12	% Hatch = 78.0	5
	Cyprinus carpio	1500 µl/l	45 min	17	% Hatch = 64	5
	Catostomus commersoni	1500 µl/l	45 min	12	% Hatch = 72	5
	Ictalurus punctatus	1500 µl/l	45 min	22	% Hatch = 100.0	5
	Acipenser fulvescens	1500 µl/l	45 min	17	% Hatch = 54.0	5
	Oncrhychus mykkis	0.1-1000 µl/l	6/24/96 hrs	12	LC_{50} = >400/220/ 21 µl/l	6
	Ictalurus punctatus	0.1-1000 µl/l	6/24/96 hrs	22	LC_{50} = 200/80/54 µl/l	6
	Coregonus albula	40-500 mg/l	24/48 hrs	13.8	LC_{50} = 133/108 mg/l	7
	Rotifers	0.5 ppm	60 min	25	% Hatch = 32	4
Eugenol	*O. mykiss*	0-30 ppm	1/3/8/96	13.9	LC_{50} = 45/17/9/9 ppm	8
SHC	Rotifers	1.0 ppm	60 min	-	% Hatch = 48	4

Table 5. Continued.

Chemical[a]	Species	Exposure			Parameter	Study[c]
		Conc.	Time	Temp. (°C)		
Hydrogen peroxide	*O. mykiss,*- Washington	1000 µl/l	15 min/day/5days	13.3	% Hatch = 75.3	9
	O. mykiss – Ganaraska	1000 µl/l	15 min/day/5days	13.3	% Hatch = 73.5	9
	O. mykiss – Skamania	1000 µl/l	15 min/day/5days	13.3	% Hatch = 28.7	9
	Salmo trutta	100-3000 µl/l	15/45 min	12	NOEC[b] = 1000/200 µl/l	10
	Salvelinus namycush	100-3000 µl/l	15/45 min	12	NOEC = 3000/1000 µl/l	10
	I. punctatus	100-3000 µl/l	15/45 min	12	NOEC = 3000/1000 µl/l	10
	Pimephales promelas	100-3000 µl/l	15/45 min	12	NOEC = 1000/500 µl/l	10
	Lepomis macrochirus	100-3000 µl/l	15/45 min	12	NOEC = 1000/100 µl/l	10
	Stizostedion vitreum	100-3000 µl/l	15/45 min	12	NOEC = 100/- µl/l	10
	O. mykiss – "sac-fry"	100-3000 µl/l	15/45 min	12	NOEC = 1000/1000 µl/l	10
	O. mykiss – "swim-up fry"	100-3000 µl/l	15/45 min	12	NOEC = 3000/500 µl/l	10
	O. mykiss – "fingerling"	100-3000 µl/l	15/45 min	12	NOEC = 500/250 µl/l	10
	O. mykiss – "small adult"	100-3000 µl/l	15/45 min	12	NOEC = 500/100 µl/l	10
	O. mykiss – "large adult"	100-3000 µl/l	15/45 min	12	NOEC = 500/250 µl/l	10
	O. mykiss	100-5000 µl/l	60 min	7/12/17/22	LC_{50} = 2380/1260/311/218 µl/l	10
	I. punctatus	100-5000 µl/l	60 min	7/12/17/22	LC_{50} = >5000/>5000/2860/2010 µl/l	10
	L. macrochirus	100-5000 µl/l	60 min	7/12/17/22	LC_{50} = 3190/2560/2180/1460 µl/l	10
	O. mykiss	100-5000 µl/l	24 hrs	7/12/17/22	LC_{50} = 69.4/42/34/31.3 µl/l	10
	I. punctatus	100-5000 µl/l	24 hrs	7/12/17/22	LC_{50} = 369/76.6/57.4/55.5 µl/l	10
	L. macrochirus	100-5000 µl/l	24 hrs	7/12/17/22	LC_{50} = 290/165/152/71.5 µl/l	10
	Esox lucius	1000 µl/l	15 min	12	% Hatch = 37.0	11
	S. vitreum	1000 µl/l	15 min	12	% Hatch = 77.0	11
	Perca flavescens	1000 µl/l	15 min	12	% Hatch = 100.0	11
	Catostomas. commersoni	1000 µl/l	15 min	12	% Hatch = 61.0	11

Table 5. Continued.

Chemical	Species	Exposure			Parameter	Study
		Conc.	Time	Temp. (°C)		
Hydrogen peroxide (cont'd)	A. fulvescens	1000 µl/l	15 min	12	% Hatch = 57.0	11
	Polyodon spathula	1000 µl/l	15 min	12	% Hatch = 82.0	11
	Cyprinus carpio	1000 µl/l	15 min	12	% Hatch = 59.0	11
	I. punctatus	1000 µl/l	15 min	22	% Hatch = 78.0	11
	O. mykiss "fry"	0-540 ppm	60 min	15	LC_{50} = 322 ppm	12
	O. mydiss "fingerlings"	0-540 ppm	60 min	15	LC_{50} = 377 ppm	12
	O. clarki "fry"	0-540 ppm	60 min	15	LC_{50} = 329 ppm	12
	O. clarki "fingerlings"	0-540 ppm	60 min	15	LC_{50} = 506 ppm	12
	Salmo salar	0-3000 µl/l	60/180 min	12	NOEC = -/- µl/l	13
	Salmo salar	0-3000 µl/l	60/180 min	12	NOEC = 221/120 µl/l	13
	Salvelinus namycush	0-3000 µl/l	60/180 min	12	NOEC = -/- µl/l	13
	Salvelinus namycush	0-3000 µl/l	60/180 min	12	NOEC = 298/113 µl/l	13
	O. mykiss	0-3000 µl/l	60/180 min	12	NOEC = 188/178 µl/l	13
	O. mykiss	0-3000 µl/l	60/180 min	12	NOEC = 79/81 µl/l	13
	E. masquinongy	0-3000 µl/l	60/180 min	17	NOEC = 104/54 µl/l	13
	E. masquinongy	0-3000 µl/l	60/180 min	17	NOEC = 104/78 µl/l	13
	E. lucius	0-3000 µl/l	60/180 min	17	NOEC = 98/54 µl/l	13
	E. lucius	0-3000 µl/l	60/180 min	17	NOEC = <76/<32 µl/l	13
	Scaphirhynchus alba	0-3000 µl/l	60/180 min	17	NOEC = <114/28 µl/l	13
	Scaphirhynchus alba	0-3000 µl/l	60/180 min	17	NOEC = 93/47 µl/l	13
	Stizostedion vitreum	0-3000 µl/l	60/180 min	17	NOEC = <72/72 µl/l	13
	Stizostedion vitreum	0-3000 µl/l	60/180 min	17	NOEC = <96/<47 µl/l	13
	C. commersoni	0-3000 µl/l	60/180 min	17	NOEC = 47/28 µl/l	13
	C. commersoni	0-3000 µl/l	60/180 min	17	NOEC = 78/47 µl/l	13

Table 5. Continued.

Chemical	Species	Exposure			Parameter	Study
		Conc.	Time	Temp. (°C)		
Hydrogen peroxide (cont'd)	*L. macrochirus*	0-3000 µl/l	60/180 min	22	NOEC = 78/47 µl/l	13
	L. macrochirus	0-3000 µl/l	60/180 min	22	NOEC = 78/47 µl/l	13
	I. punctatus	0-3000 µl/l	60/180 min	22	NOEC = 78/28 µl/l	13
	I. punctatus	0-3000 µl/l	60/180 min	22	NOEC = 78/47 µl/l	13
	P. promales	0-3000 µl/l	60/180 min	22	NOEC = 47/28 µl/l	13
	P. promales	0-3000 µl/l	60/180 min	22	NOEC = 78/47 µl/l	13
	Micropterus salmoides	0-3000 µl/l	60/180 min	22	NOEC = 179/91 µl/l	13
	Micropterus salmoides	0-3000 µl/l	60/180 min	22	NOEC = 130/47 µl/l	13
	Perca flavescens	0-3000 µl/l	60/180 min	22	NOEC = <47/42 µl/l	13
	Perca flavescens	0-3000 µl/l	60/180 min	22	NOEC = <130/78 µl/l	13

* - not reported/no data.

[a] OTC – oxytetracycline; SDZ - sulfadiazine; TMP - trimethoprim; SHC – sodium hypochlorite

[b] NOEC – no observable effect concentration

[c] (1) Wollenberger et al. 2000; (2) Baguer et al. 2000; (3) Holten Lützhøft et al. 1999; (4) Balompapueng et al. 1997; (5) Rach et al. 1997b; (6) Howe et al. 1995; (7) Ritola and Lyytikainen 1995; (8) Keene et al. 1998; (9) Gaikowski et al. 1998; (10) Rach et al. 1997a; (11) Rach et al. 1998; (12) Arndt and Wagner 1997; (13) Gaikowski et al. 1999.

Table 6. Summary of results reported in studies examining the impact of antibiotics on bacterial resistance. Background resistance refers to resistance levels present in under-cage sediment prior to antibiotic therapy, resistance in a non-farm site, or in control mesocosms, depending on the circumstances of the study. Maximum resistance is the highest level of resistance observed for an experimental unit subject to antibiotic therapy. Time to return to background refers to the number of days post-therapy for bacterial resistance levels to return to pre-treatment, off-site, or control levels of resistance. In Samuelsen et al.(1992b), time to return to background refers to the number of days to reach minimum resistance levels.

	Antibiotic*	**Background Resistance**[a]	**Maximum Resistance**[a]	**Time to Return to Background**
McPhearson et al. 1991	OTC	0.8	69.1	-
Spanggaard et al. 1993	OTC	6	15	-
Hameed et al. 2003	OTC	-	95	-
Bjorklund et al. 1991	OTC	-	0.94 µg/l[b]	-
Miranda and Zemelman 2002a	OTC	-	64-2048 µg/l[b]	-
Samuelsen et al. 1992b	OTC	-	140	80 days
Herwig et al. 1997	OTC	3	20	-
	SMO	1	9	-
Bjorklund et al. 1990	OTC	1.6-1.7 µg/l[b]	4.7-18.8 µg/l[b]	-
Ervik et al. 1994	OTC	0.6	46	-
Nygaard et al. 1992	OTC	5	16	-
Kerry et al. 1994	OTC	0.3	16	73 days
Kerry et al. 1996a	$OTC_{0.1\text{-}6.25\ \mu g/l}$	0.6	1.2-4.6	-
	$OTC_{12.5\text{-}300\ \mu g/l}$	0.6	86.9-95.8	-
	$OTC_{400\text{-}600\ \mu g/l}$	0.6	108	-
Kerry et al. 1996b	$OTC_{25\ \mu g/l}$	0.4	35.1	-
	$OTC_{100\ \mu g/l}$	1.3	25.4	-
Kerry et al. 1997	OTC_{gut}	4.7	10	-
	OTC_{water}	1	27	-
Herwig and Gray 1997	OTC	5.0	45	-
	SMO	5.0	>100	-
Bruun et al. 2000	OTC	0.063-0.13	6.4 µg/l[b]	-
	FLO	0.5-1.0	0.61 µg/l[b]	-
	SDT	16-32	113 µg/l[b]	-
Kim and Aoki 1996	OTC	0.2-0.5	50 µg/l[b]	-
	FLO	0.1-0.4	0.4 µg/l[b]	-
	TRM	0.8	25 µg/l[b]	-
Rangdale et al. 1997	OTC	0.125	8/32 µg/l[b, c]	-
	FLO	0.5	1.0/8.0 µg/l[b, c]	-
	SMO	16	64/256 µg/l[b, c]	-

Table 6. Continued.

	Antibiotic*	Background Resistance[a]	Maximum Resistance[a]	Time to Return to Background
Rodgers 2001	OTC	-	2 μg/l[b]	-
	SMO	-	64 μg/l[b]	-
Vaughan et al. 1996	OTC	0.25-1.2	41-43	-

* OTC – oxytetracycline; SMO - sufadimethoxine/ormetoprim; SDT - sulfadiazine trimethoprim; TRM - trimethoprim; FLO - florfenicol

[a] Resistance is reported as ((# colonies growing on antibiotic containing medium)/(# colonies growing on medium without antibiotic)) * 100%, unless noted otherwise.

[b] Values refer to the minimum inhibitory concentration of antibiotic in the culture medium.

[c] Values are MIC_{50}/MIC_{90} where MIC_x refers to MIC required to inhibit growth of x% of isolates

Table 7. Summary of plating media used in the studies reviewed.

	Medium[a]	Drug[b]	Concentration (μg/ml)	Other additions
McPherson et al. 1991	Mac	OTC	30	
Spanggaard et al. 1993	IA	OTC	8	
Bjorklund et al. 1991	SP	OTC	?	
Samuelsen et al. 1992b	MA	OTC	25	
	TSCA	OTC	25	
Kerry et al. 1994	ZV	OTC	25	
Herwig et al. 1997	MH	OTC	30	Sea salts
	MH	SMO	23.8/1.2 [c]	Sea salts
Bjorklund et al. 1990	TSA	OTC	10	
	TCBS	OTC	10	
Ervik et al. 1994	TSA	OTC	100	70% Seawater
Nygaard et al. 1992	TSA	OTC	25	70% Seawater
Kerry et al. 1996	ZV	OTC	0-600	
Herwig and Gray 1997	MH	OTC	90	Sea salts
	MH	SMO	25/5 [c]	Sea salts
O'Reilly and Smith 2001	ISA	OTC	0.1-200	
Vaughan et al. 1996	CPSA	OTC	25	
Bruun et al. 2000	MH	OTC	0.016-256	1.5% Select agar
	MH	FLO	0.016-256	1.5% Select agar
	MH	SDT	0.016-256	1.5% Select agar
Kim and Aoki 1993	MH	OTC	25	2% NaCl
	MH	FLO		2% NaCl
	MH	TRM	112.5	2% NaCl
Hameed et al. 2003	MH	OTC	30	Seawater
Miranda and Zemelman 2002	TSA	OTC	30/100 [d]	
Ho et al. 2000	MH	OTC	0.002-100	
	MH	FLO	0.002-100	
Rodgers 2001	TSA	OTC	0.00012-256	
	TSa	SDT	0.00012-256	
Kerry et al. 1996b	ZV	OTC	25/100 [d]	
Kerry et al. 1997	ZV	OTC	25	

[a] Mac – MacConkey Agar; IA – Aron Agar ; LB – Luria Bertani Agar ; SP – Sensititre Plates, Gibco; TSA – Trptone Soya Agar; MA- Marine Agar; TSCA – Tryptone Soya Agar plus Sodium Citrate; ZV- 2216 Z Agar; MH – Mueller-Hinton Agar; TCBS – Thiosulfate-Citrate-Bile-Sucrose Agar; ISA – Iso-Senstitest Agar; CPSA – Casein Peptone Starch agar.

[b] OTC - oxytetracycline; SMO sulfamethoxine ormetorpim; FLO - florefenicol; SDT - sulfadiazine trimethoprim; TRM - trmimethprim

[c] ratio of sulfamethoxine/ormetoprim by mass

[d] two concentrations of OTC were used